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CULTURE OF HUMAN MARROW

A COMPARATIVE STUDY OF THE EFFECTS OF SULFANILAMIDE AND ANTIPNEUMOCOCCUS SERUM ON THE COURSE OF EXPERIMENTAL PNEUMOCOCCIC INFECTIONS

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The pneumococcus was chosen for this study because of the great clinical importance of pneumococcic infections and because the studies that have been published have given conflicting and inconclusive results.

REVIEW OF THE LITERATURE

The vaccine vial technic of culture of human marrow [1] permits quantitative studies of the interaction of living human cells and noxious and therapeutic agents. Studies of the mode of action of sulfanilamide on experimental infections due to beta hemolytic streptococci [2] indicated that sulfanilamide itself did not kill the organisms but rendered them vulnerable to bactericidal substances present in normal human serum, probably by preventing the formation, or by neutralization, of the toxins or aggressins of the organisms. These studies also indicated that the concentration of sulfanilamide necessary to control hemolytic streptococcic infections in the cultures was about 1 : 100,000 instead of 1 : 10,000, which had previously been recommended, and that even short periods without sulfanilamide led to rapid multiplication and the production of toxin. In the few months since that article was published much clinical evidence has accumulated to indicate that the conclusions derived from these in vitro experiments are clinically applicable. For example, from 0.3 to 0.6 Gm. of sulfanilamide every four hours, day and night, has proved more effective against hemolytic streptococcic infections than a much greater daily dose of sulfanilamide given in three doses.

From the Department of Medicine and the Division of Experimental Medicine, University of Oregon Medical School.

1. Osgood, E. E., and Brownlee, I. E.: Culture of Human Marrow: Details of a Simple Method, J. A. M. A. **108**:1793 (May 22) 1937.

2. Osgood, E. E.: Culture of Human Marrow: Studies on the Mode of Action of Sulfanilamide, J. A. M. A. **110**:349 (Jan. 29) 1938.

A little work has been done on the action of sulfanilamide in pneumococcic infections. The in vitro [3] studies require confirmation. Mice, rats and rabbits with pneumococcic infections have been treated with doses of sulfanilamide so large,[4] corresponding to from 30 to 125 Gm. a day for a man, that although survivals have occurred, it is difficult to see what bearing these results may have on treatment of human beings with pneumococcic infections. When doses comparable to those feasible for human beings [5] have been used, delayed death has been the usual result. Heintzelman, Hadley and Mellon [6] have reported a reduction in the mortality of patients with pneumonia due to type III pneumococcus from 73 per cent for the controls to 22 per cent with sulfanilamide therapy for a small series of cases, and a few other cases of recovery from pneumonia after treatment with sulfanilamide have been reported without controls.[7] A few cases of pneumococcic meningitis have been reported in which recovery occurred [8] after the use of sulfanilamide, although in most such cases [9] death was only delayed. Little

3. Rosenthal, S. M.: Studies in Chemotherapy: III. The Effect of *p*-Aminobenzene Sulphonamide on Pneumococci in Vitro, Pub. Health Rep. **52**: 192, 1937.

4. (*a*) Rosenthal, S. M.: Studies in Chemotherapy: II. The Chemotherapy of Experimental Pneumococcus Infections, Pub. Health Rep. **52**:48, 1937. (*b*) Cooper, F. B.; Gross, P., and Mellon, R. R.: Action of *p*-Aminobenzenesulfonamide on Type III Pneumococcus Infections in Mice, Proc. Soc. Exper. Biol. & Med. **36**:148, 1937. (*c*) Rosenthal, S. M.; Bauer, H., and Branham, S. E.: Studies in Chemotherapy: IV. Comparative Studies of Sulphonamide Compounds in Experimental Pneumococcus, Streptococcus, and Meningococcus Infections, Pub. Health Rep. **52**:662, 1937. (*d*) Cooper, F. B., and Gross, P.: Para-Aminobenzenesulfonamide Therapy in Experimental Type III Pneumococcal Pneumonia, Proc. Soc. Exper. Biol. & Med. **36**:678, 1937. (*e*) Kreidler, W. A.: Treatment of Pneumococcal Infections in Rabbits with Sulfanilamide, ibid. **37**: 205, 1937. (*f*) Schmidt, L. H.: Use of Sulfanilamide in the Treatment of Type XIV Pneumococcus Infections in Mice, ibid. **37**:205, 1937. (*g*) Buttle, G. A. H.: Discussion on the Treatment of Bacterial Diseases with Substances Related to Sulphanilamide, Proc. Roy. Soc. Med. **31**:154, 1937. (*h*) Bauer, H., and Rosenthal, S. M.: Studies in Chemotherapy: VII. Some New Sulphur Compounds Active Against Bacterial Infections, Pub. Health Rep. **53**:40, 1938.

5. Buttle, G. A. H.; Parish, H. J.; McLeod, M., and Stephenson, D.: The Chemotherapy of Typhoid and Some Other Non-Streptococcal Infections in Mice, Lancet **1**:681, 1937.

6. Heintzelman, J. H. L.; Hadley, P. B., and Mellon, R. R.: The Use of *p*-Aminobenzenesulphonamide in Type III Pneumococcus Pneumonia, Am. J. M. Sc. **193**:759, 1937.

7. Millett, J.: The Action of Sulfanilamide in a Case of Type III Pneumococcus Pneumonia, New York State J. Med. **37**:1743, 1937.

8. Mertins, P. S., and Mertins, P. S., Jr.: Meningitis Due to the Type IV Pneumococcus, with Recovery: Report of a Case, Arch. Otolaryng. **25**:657 (June) 1937.

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work has been reported on the combined use of sulfanilamide and antipneumococcus serum.[10]

It is evident that if the value of sulfanilamide or of combinations of sulfanilamide and antiserum is to be determined by clinical investigation, a carefully controlled study of a large series of cases would have to be made, as was done in evaluating antipneumococcus serums.[11] Such studies are practicable only in large hospitals and involve some risk for the patients who receive the therapy which ultimately proves to be unsatisfactory. It is hoped that results of investigations of marrow culture such as are reported in this article will serve as a guide to the most profitable line of clinical investigation. It remains to be seen whether the data derived from such studies will be confirmed by clinical investigation. If so, this simple and accurately controllable procedure may ultimately prove a valuable supplement to animal experimentation and clinical investigation.

METHOD

The method of culturing human marrow has been described.[2] Briefly, our procedure has been to introduce about 10 cc. of sternal marrow into a 50 cc. vaccine vial containing about 25 cc. of citrated balanced salt solution. By centrifugation and removal of the buffy coat, the nucleated cells of the marrow are separated from the akaryocytes (non-nucleated erythrocytes) and diluted in a volume of marrow culture medium[12] so that the total nucleated cell count is between 1,000 and 2,000 per cubic millimeter. This culture is then brought to incubator temperature, and a suitable dilution of an actively growing virulent culture of the desired type of pneumococcus is added, the culture is thoroughly shaken and a portion is removed for the initial blood agar pour plate colony count.

9. Long, P. H.: The Treatment of Certain Infections with Sulphanilamide or Its Derivatives, Internat. S. Digest **23**:259, 1937. Millett, J.: The Intrathecal Use of Prontosil Soluble: Report of a Case of Type III Pneumococcus Meningitis and Septicemia Treated with Prontosil Soluble, with Complete Autopsy Report, J. A. M. A. **109**:2138 (Dec. 25) 1937.

10. Branham, S. E., and Rosenthal, S. M.: Studies in Chemotherapy: V. Sulphanilamide, Serum, and Combined Drug and Serum Therapy in Experimental Meningococcus and Pneumococcus Infections in Mice, Pub. Health Rep. **52**:685, 1937. Gross, P., and Cooper, F. B.: *p*-Aminobenzenesulfonamide and Antipneumococcal Serum Therapy in Type I Pneumococcal Infections of Rats, Proc. Soc. Exper. Biol. & Med. **36**:535, 1937. Cooper, F. B., and Gross, P.: Sulfanilamide, Antipneumococcus Serum and Vitamin C Therapy in Type II Pneumococcal Pneumonia of Rats, ibid. **36**:774, 1937. Brown, A. E.; Bannick, E. G., and Habein, H. C.: The Use of Sulfanilamide and Prontosil Solution, Minnesota Med. **20**: 691, 1937.

11. Cecil, R. L., and Plummer, N.: Pneumococcus Type I Pneumonia: A Study of Eleven Hundred and Sixty-One Cases, with Especial Reference to Specific Therapy, J. A. M. A. **95**:1547 (Nov. 22) 1930. Bullowa, J. G. M., and Wilcox, C.: Endemic Pneumonia: Pneumococcic Types and Their Variations in Incidence and Mortality for Adults and Children, Arch. Int. Med. **59**:394 (March) 1937.

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The thoroughly mixed culture is then divided into five to seven vaccine vials, 8 cc. being placed in each. One of these vials is kept as a control. To the others the desired quantity of sulfanilamide or antipneumococcus serum diluted in balanced salt solution is added, and to the control an equal amount of balanced salt solution is added. The content of each vial is thoroughly mixed, and all are placed in an incubator. The time recorded is the time from the introduction of the cultures into the incubator, which is usually about fifteen minutes after the sample is withdrawn for the initial colony count. From this point on, all the cultures in one experiment are handled identically. Equal portions are removed at intervals for the making of colony counts, cell counts and smears (Wright's stain). This technic insures that all the cultures in one experiment contain the same number and type of cells, the same volume and composition of the medium and the same initial

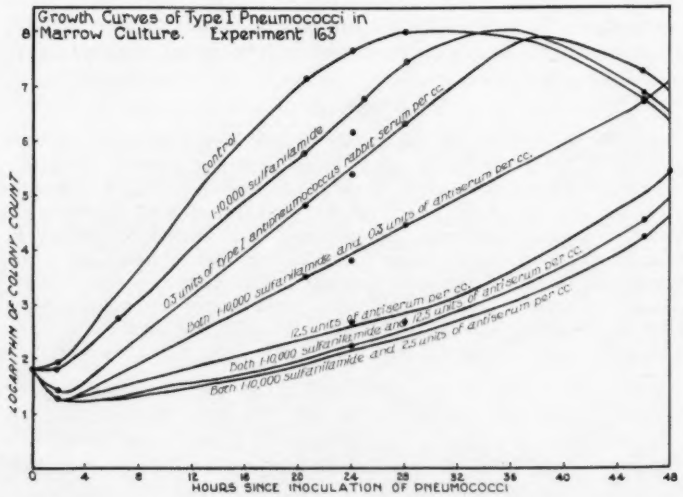
Growth Curves of Type I Pneumococci in Marrow Culture. Experiment 163
LOGARITHM OF COLONY COUNT
HOURS SINCE INOCULATION OF PNEUMOCOCCI
Control
1:10,000 sulfanilamide
0.3 units of type I antipneumococcus rabbit serum per cc.
Both 1:10,000 sulfanilamide and 0.3 units of antiserum per cc.
12.5 units of antiserum per cc.
Both 1:10,000 sulfanilamide and 12.5 units of antiserum per cc.
Both 1:10,000 sulfanilamide and 2.5 units of antiserum per cc.

Fig. 1.—Logarithmic growth curves obtained in a typical experiment. By seventy hours the curve for cultures containing sulfanilamide plus 2.5 units of antiserum had risen to 600,000, and the curve for 12.5 units of antiserum plus sulfanilamide had risen to 300,000 colonies per cubic centimeter. In many of the experiments these curves decreased to zero by twelve to twenty-four hours.

inoculation of pneumococci. In other words, all factors are identical except the single variable that is purposely introduced. This is a type of control which is impossible to secure in investigations on human beings or animals.

12. This consists of 35 per cent human cord serum and the Gey and Gey (Gey, G. O., and Gey, M. K.: The Maintenance of Human Normal Cells and Tumor Cells in Continuous Culture: I. Preliminary Report; Cultivation of Mesoblastic Tumors and Normal Tissue and Notes on Methods of Cultivation, Am. J. Cancer **27**:45, 1936) balanced salt solution or the patient's own plasma and citrated balanced salt solution.

RESULTS

Controls.—The course of the growth curve in a typical experiment is shown in figure 1. In general, the larger the initial inoculation of pneumococci, the more steeply the curve rises, the earlier the peak is reached and the earlier the phase of decrease in counts begins. With the largest amount of inoculation employed, 750 organisms per cubic centimeter, a peak of about 100,000,000 was reached in sixteen hours,

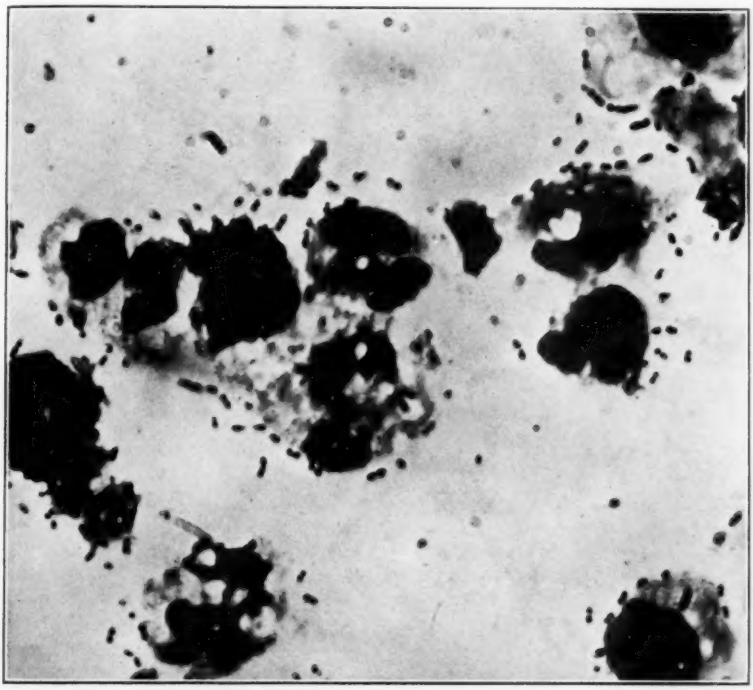

Fig. 2.—Photomicrograph of a smear made at nineteen hours for the control in experiment 146. The initial inoculation was of 700 type II pneumococci per cubic centimeter. Compare with figures 3 and 4. Wright's stain; × 2,000.

while with inoculations of under 50 organisms per cubic centimeter, the peak was not reached for twenty-eight to thirty-two hours.

On microscopic examination the number of organisms appeared to increase even after the growth curves had begun to subside, indicating that dead organisms were visible. Smears (Wright's stain) from forty-eight to seventy-two hour cultures showed large masses of organisms and much damaged marrow cells. However, in the twenty-four hour cultures both leukocytes and erythrocytes were relatively well preserved (figs. 2 and 5), in strong contrast to the picture revealed by studies of

the beta hemolytic streptococcus,[2] in which there was complete destruction of all marrow cells in the controls in this period.

Control studies were made of pneumococci of types I, II, III and VIII and of group E (not further identified). With each, the course of the infection was much the same. In most marrow cultures the pneumococci were almost entirely extracellular (fig. 2), but in a few, phagocytosis occurred in the controls. The phagocytosis in the controls

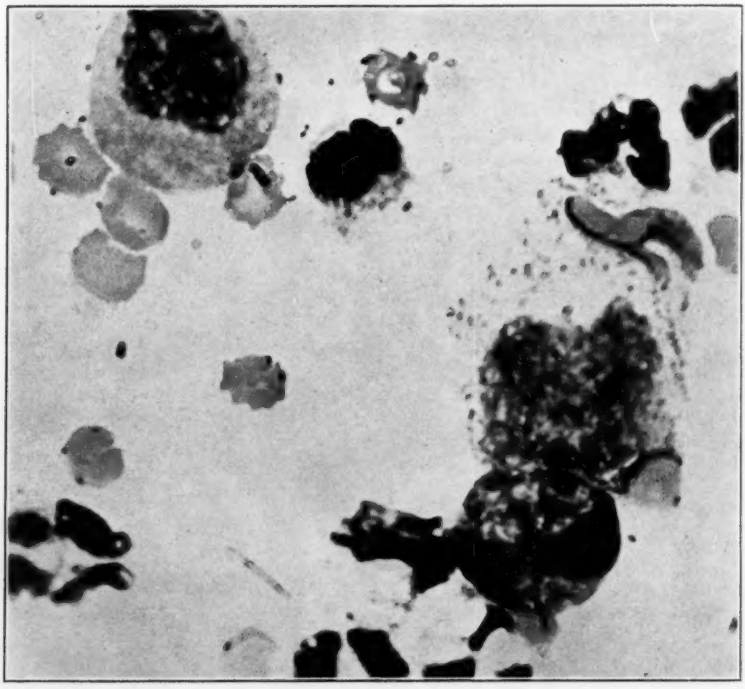

Fig. 3.—Photomicrograph of a smear made at nineteen hours of the culture containing 1 : 100,000 sulfanilamide in experiment 146 in which 700 type II pneumococci were initially inoculated. Compare with figures 2 and 4. Note the absence of phagocytosis and the good structure of the marrow cells. Wright's stain; × 2,000.

seemed to be due to a variation in the serum or plasma used for the culture medium rather than in the marrow cells. In most cultures the organisms retained their typical structure and remained encapsulated. In a few cases, in all of which phagocytosis occurred, there were bizarre, morphologic alterations, with little apparent effect, however, on the course of the growth curve.

Sulfanilamide.—In preliminary experiments 1 : 1,000, 1 : 10,000 and 1 : 100,000 concentrations of sulfanilamide [13] were used in studying infections with pneumococci of types I, II, III and VIII and of group E. Bacteriostasis increased with increase in the concentration of sulfanilamide (figs. 3 and 4). Thereafter, most of the experiments with sulfanilamide alone were done with the 1 : 10,000 concentration, since 1 : 100,000 was much less effective and 1 : 1,000, while more effective,

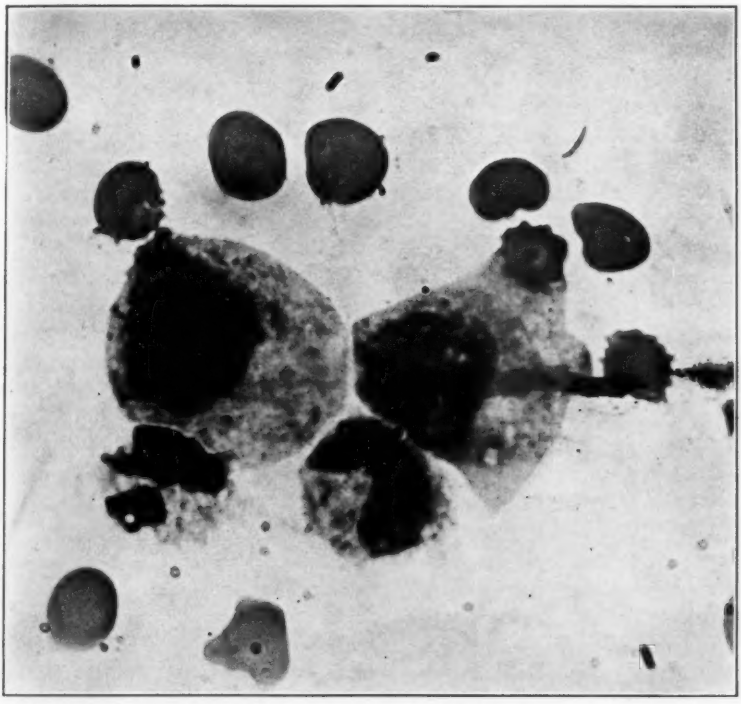

Fig. 4.—Photomicrograph of a smear made at nineteen hours from the culture containing 1 :10,000 sulfanilamide in experiment 146. The initial inoculation was of 700 type II pneumococci per cubic centimeter. Compare with figures 2 and 3. No pneumococci are visible in this field, but on prolonged search through many fields occasional typical extracellular diplococci were found. Wright's stain; × 2,000.

would require for human beings a dose of sulfanilamide of over 70 Gm. a day, which appears to be dangerous. In all the experiments performed, comparison of smears made between twelve and twenty-eight hours showed fewer organisms and better looking cells in the cultures contain-

13. The sulfanilamide used was supplied by the Winthrop Chemical Co., Inc.

ing sulfanilamide (figs. 3, 4 and 6) than in the corresponding controls (figs. 2 and 5) for any type of pneumococci studied.

A typical comparative growth curve is shown in figure 1. In none of the experiments, no matter how small the initial inoculation or how great the concentration of sulfanilamide up to 1 : 1,000, did the culture become sterile, and as far as was determined the peak was of the same general order as for the controls, but it took longer for this peak to be reached. There was no evidence of any effect of sulfanilamide on phagocytosis. If there was no phagocytosis in the control culture, there was none in the corresponding preparation containing sulfanilamide. If there was phagocytosis in the culture containing sulfanilamide, it was present in the corresponding control. The structure of the organisms appeared the same as that of the organisms in the control culture containing a corresponding number of organisms. Confirming the reports of Long [14] and of Colebrook and Kenny,[15] capsules were not dissolved, as has been claimed by Levaditi and Vaisman,[16] nor were the capsules swollen, as they were in the cultures containing rabbit antipneumococcus serum. In other words, slight bacteriostasis was the only effect of sulfanilamide on pneumococcic infections in marrow cultures; whereas, in infections in marrow cultures due to beta hemolytic streptococci,[2] because of the presence of bactericidal substances in the serum used in the marrow culture medium, complete sterilization ensued. This suggests that substances bactericidal for the pneumococcus are not present in most human serums and that the effects of antipneumococcus serum in combination with sulfanilamide should be investigated. The effects of the antiserum alone also were determined to serve as a control on the effects of the two together.

Antipneumococcus Serum.—The experiments with antipneumococcus serum were done with a strain of type I pneumococcus which was highly virulent for mice, since antipneumococcus serum has proved most effective clinically against type I pneumococcus. Both horse and rabbit antiserum [17] were used, but no attempt was made to compare their effectiveness. All the antiserum used in any one experiment was from

14. Long, P. H., and Bliss, E. A.: Para-Amino-Benzene-Sulphonamide (Sulphanilamide) or Its Derivatives in the Treatment of Infections Due to Beta-Haemolytic Streptococci, Pneumococci and Meningococci, South. M. J. **30**:479, 1937.

15. Colebrook, L., and Kenny, M.: Treatment of Human Puerperal Infections, and of Experimental Infections in Mice, with Prontosil, Lancet **1**:1279, 1936.

16. Levaditi, C., and Vaisman, A.: Action curative du chlorhydrate de 4'-sulfamido-2,4-diaminoazobenzène et de quelques dérivés similaires, dans la streptococcie expérimentale, Compt. rend. Soc. de biol. **119**:946, 1935.

17. The horse antiserum was Mulford type I, sold by Sharp and Dohme. Eli Lilly & Co. supplied the rabbit antipneumococcus serum (lot B4454) used, which is not yet available for commercial distribution.

one source. One difference noted between them was the absence with horse antiserum of the marked swelling of the capsules that was uniformly present when rabbit antiserum was used. The only other difference noted was the presence of a prozone phenomenon [18] with horse antiserum so that doses above 3 units per cubic centimeter were not as effective as smaller doses; with the rabbit antiserum an increase in dose resulted in a greater or, in the case of a very high dose, in an equal

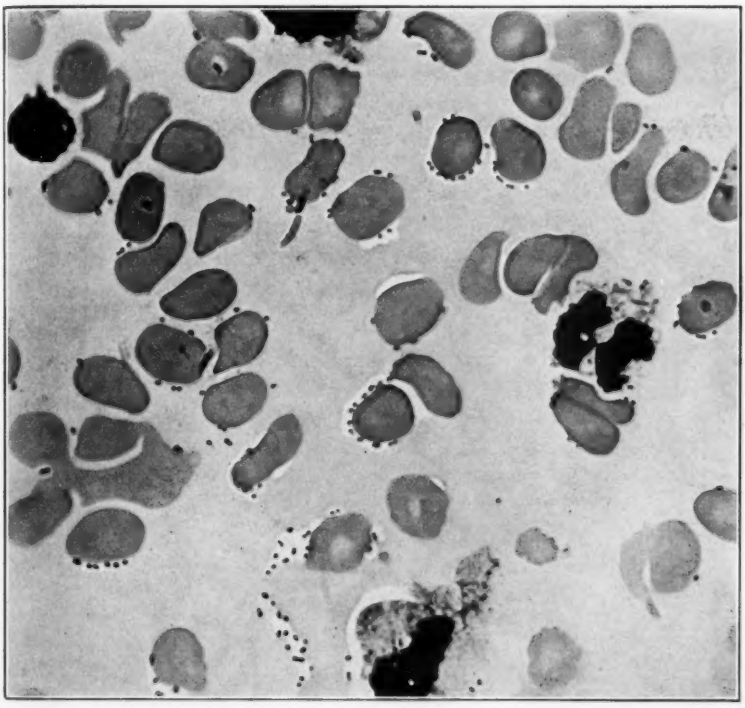

Fig. 5.—Photomicrograph made at twenty-fours of a smear from the control culture in experiment 158 in which the initial inoculation of type I pneumococci was 80 per cubic centimeter. Compare with figures 6 to 8. Note the large number of typical encapsulated extracellular diplococci and the slight damage to the marrow cells. Wright's stain; × 1,000.

effectiveness. In all cultures containing antiserum (fig. 7), even though the dose was as small as 0.03 unit per cubic centimeter, a great increase in phagocytosis was noted, few organisms being found extracellularly. This

18. Goodner, K., and Horsfall, F. L., Jr.: The Protective Action of Type I Antipneumococcus Serum in Mice: I. The Quantitative Aspects of the Mouse Protection Test, J. Exper. Med. **62**:359, 1935.

was most striking when there was little or no phagocytosis in the corresponding controls and in the cultures containing sulfanilamide. In the same experiment, if two different concentrations of antiserum were used, the growth curves followed each other closely for the first few hours, but the curve for the culture containing the smaller concentration rose earlier. As a rule there was a definite drop in the number of organisms in the first few hours, even with the lowest concentrations, which was

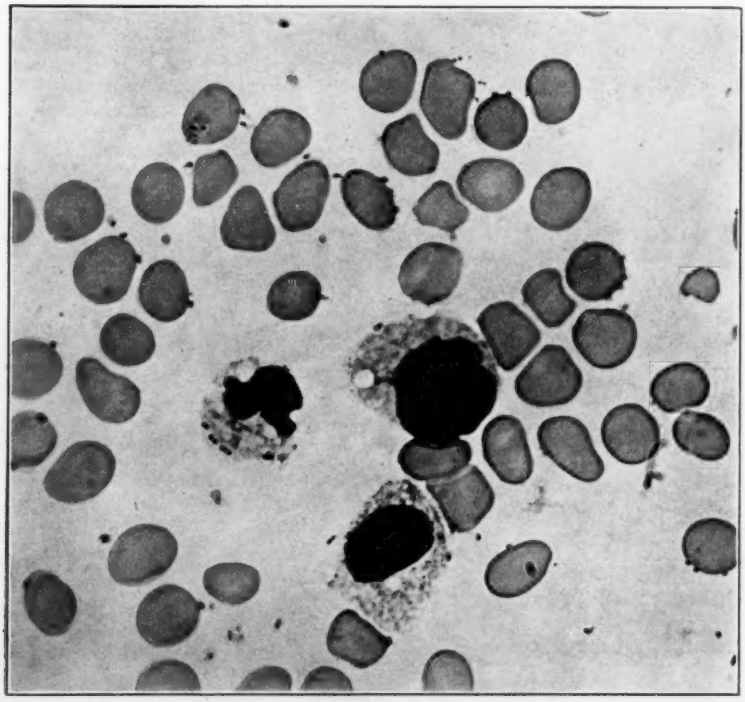

Fig. 6.—Photomicrograph of a smear made at twenty-four hours from the culture containing 1:100,000 sulfanilamide in experiment 158 in which the initial inoculation was of 80 type I pneumococci per cubic centimeter. Compare with figures 5, 7 and 8. Note the much smaller number of pneumococci than in the smear for the control and the extracellular position of the organisms. Note also that capsules are still present and that the structure of the marrow cells is good. Wright's stain; × 1,000.

not noted for the controls or for the cultures containing sulfanilamide alone. How much of this drop was due to phagocytosis and agglutination and how much to actual killing of the organisms was not determined. The smallest quantity of antiserum used, 0.03 units per cubic

centimeter of horse antiserum, had a somewhat slighter effect on the colony counts than 1 : 10,000 sulfanilamide in the corresponding cultures, but the next smallest dose used, 0.3 unit per cubic centimeter of horse or rabbit antiserum, was definitely more effective than 1 : 10,000 sulfanilamide in the corresponding cultures (fig. 1). Even the highest concentration used, 50 units per cubic centimeter of rabbit antiserum, rarely sterilized cultures completely, although as a rule only a single

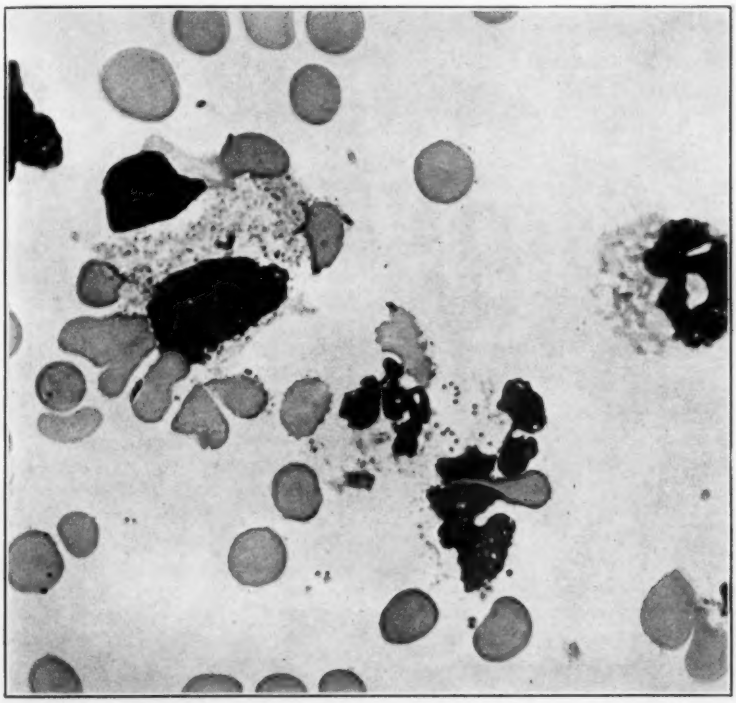

Fig. 7.—Photomicrograph of a smear made at twenty-four hours from the culture containing 0.6 unit of rabbit antiserum per cubic centimeter in experiment 158. The initial inoculation was of 80 type I pneumococci per cubic centimeter. Compare with figures 5, 6 and 8. Note the small number of pneumococci present, the intracellular location and the marked swelling of the capsules. A number of fields had to be examined to find any pneumococci. Wright's stain; × 1,000.

colony was found occasionally in pour plates made with 0.5 cc. of undiluted culture.

Concentrations of 0.3, 0.6 and 1.0 unit per cubic centimeter showed an increase in the count after the first four hours in all experiments in which they were used, the rate of increase being less for a longer period

in the cultures containing the higher concentrations. The peak colony counts for these cultures were usually lower than those reached in the corresponding control and sulfanilamide experiments, and a longer time was required to reach the peak. None of the cultures containing these concentrations became sterile. The same applied to most of the cultures with 2.5 and 12.5 units per cubic centimeter, with the exception of experiments 166 and 167, which will be discussed in detail later. For

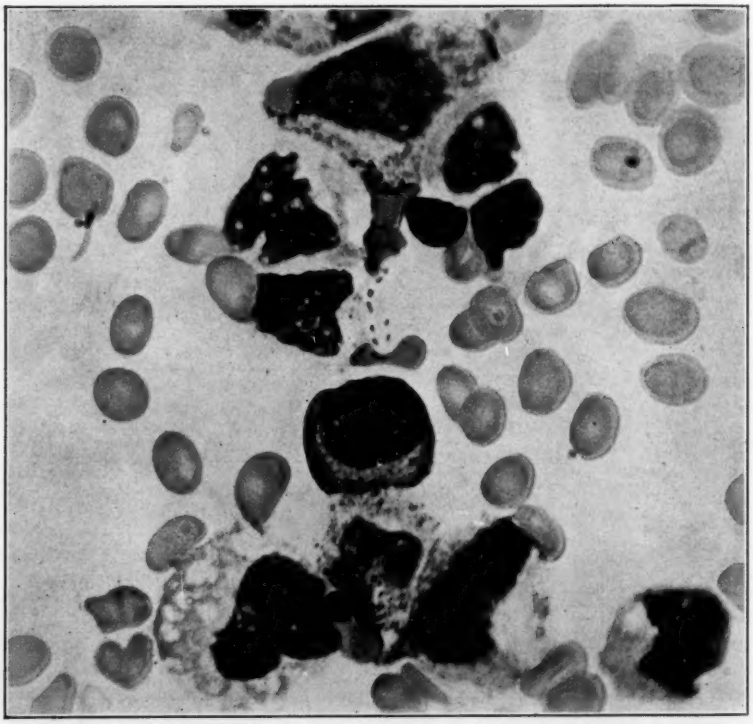

Fig. 8.—Photomicrograph of a smear made at twenty-four hours from a culture containing both 1:10,000 sulfanilamide and 0.6 unit of rabbit antiserum per cubic centimeter in experiment 158. The initial inoculation was of 80 type I pneumococci per cubic centimeter. Compare with figures 5 to 7. Note the few extracellular pneumococci with swollen capsules in the leukocyte in the center of the field. A prolonged search was necessary to find any pneumococci at all in this culture. Note also the well preserved structure of the marrow cells. Wright's stain; × 1,000.

examples of the effects of various concentrations of serum alone, see figure 1 and experiments 151, 155, 164 and 165, given under the discussion concerning the use of sulfanilamide plus antiserum.

Sulfanilamide Plus Antipneumococcus Serum.—In all the experiments except experiments 166 and 167, in which sulfanilamide was used in combination with antiserum, the growth curves were significantly lower than the corresponding curves for sulfanilamide alone or for the same dose of antiserum alone. Typical examples are shown in figure 1, where it will be noted that 1:10,000 sulfanilamide plus 0.3 unit of rabbit antiserum per cubic centimeter is far more effective than this concentration of either sulfanilamide or of rabbit antiserum alone and that 1:10,000 sulfanilamide plus 2.5 units of rabbit antiserum is more effective than five times as much antiserum alone. Even with the smaller doses of antiserum plus sulfanilamide the peak colony count was not nearly as high as for the corresponding cultures containing the same amount of antiserum alone, although as a rule there was no significant difference between the colony counts for the first four to six hours. The following experiments illustrate typical effects of sulfanilamide with antiserum. In all instances the organism was type I pneumococcus, and the concentration of sulfanilamide was 1:10,000. In experiments 151 and 155 horse antiserum was used, and in experiments 164 and 165 rabbit antiserum was used. The figures indicate colonies per cubic centimeter.

Experiment 151					
Hours	0		6		26
Sulfanilamide	130		600		<1,000,000
Antiserum, 0.03 unit per cc.	130		300		600,000
Antiserum, 0.03 unit per cc. plus sulfanilamide	130		300		70,000
Experiment 155					
Hours	0		2		24
Control	200		250		<50,000,000
Antiserum, 0.03 unit per cc.	200		180		12,000,000
Antiserum, 0.03 unit per cc. plus sulfanilamide	200		180		700,000
Experiment 164					
Hours	0		16		23
Control	20		60,000,000		40,000,000
Sulfanilamide	20		25,000,000		
Antiserum, 2.5 units per cc.	20		.....		22,000,000
Antiserum, 2.5 units per cc. plus sulfanilamide	20		.....		1,300,000
Antiserum, 12.5 units per cc.	20		.....		13,000,000
Antiserum, 12.5 units per cc. plus sulfanilamide	20		.....		13,000
Experiment 165					
Hours	0	18	24	43	70
Antiserum, 2.5 units per cc.	6	2,500	30,000	175,000	100,000
Antiserum, 2.5 units per cc. plus sulfanilamide	6	10	30	15	4,000
Antiserum, 12.5 units per cc.	6	60	1,300	35,000	15,000
Antiserum, 12.5 units per cc. plus sulfanilamide	6	20	0	0	0

For no culture with an initial count under 750 per cubic centimeter containing both sulfanilamide and antiserum in concentrations of 5 or more units per cubic centimeter was there recorded any colony count above 20,000 per cubic centimeter in the first twenty-four hours, and in the majority of instances in which 12.5 units per cubic centimeter was used, the count did not go above 100 or 200 per cubic centimeter, several being sterile with many 0.2 or 0.5 cc. inoculations. However, in all cases colonies were found on pour plates made at some time between sixteen and seventy-two hours after direct inoculation with 0.5 cc.

To determine whether the action of the antiserum was specific and in order to provide further control, one experiment was performed in which the effects on the type I pneumococcus of 12.5 units per cubic centimeter of type I or type II rabbit antiserum either with or without 1:10,000 sulfanilamide were compared. The initial count was 750 per cubic centimeter. At sixteen and one-half hours the control culture showed 130,000,000, the culture containing sulfanilamide alone showed more than 500,000, that containing type I antiserum showed 28,000, that containing type I antiserum plus sulfanilamide showed 9,000, that containing type II antiserum showed more than 500,000 and that containing type II antiserum plus sulfanilamide showed 75,000 colonies per cubic centimeter. It was evident from this experiment that the type II antiserum had some effect but was far less effective than the specific antiserum, so no further work was done with type II antiserum for type I infections.

Two experiments (experiments 166 and 167) with the type I pneumococcus and rabbit antiserum gave atypical results and are reported in detail. In experiment 166, 5 indicates 1 colony from an inoculation of 0.2 cc., and 2 indicates 1 colony from an inoculation of 0.5 cc.

Experiment 166

Hours	0	4	8	12	16	20	24	48
Control	250	800	42,000	900,000	12,000,000	42,000,000	70,000,000	450,000
Sulfanilamide	250	800	36,000	700,000	2,000,000	50,000,000	72,000,000	300,000
Antiserum, 2.5 units per cc.	250	30	0	5	0	0	0	0
Antiserum, 2.5 units per cc. plus sulfanilamide	250	30	5	0	0	0	0	0
Antiserum, 12.5 units per cc.	250	30	0	5	0	0	0	0
Antiserum, 12.5 units per cc. plus sulfanilamide	250	40	40	0	5	0	0	2

Experiment 167

Hours	0	2	12	16	20	36	40	44
Control	280	350	750,000	13,000,000	?	4,000,000	1,600,000	300,000
Sulfanilamide	280	290	800,000	7,000,000	50,000,000	6,000,000	1,800,000	100,000
Antiserum, 0.3 unit per cc.	280	180	4,000	20,000	200,000	50,000,000	18,000,000	2,400,000
Antiserum, 0.3 unit per cc. plus sulfanilamide	280	170	3,000	12,000	75,000	?	?	6,500,000
Antiserum, 2.5 units per cc.	280	150	850	300	500	170,000	650,000	800,000
Antiserum, 2.5 units per cc. plus sulfanilamide	280	160	3,000	500	500	?	90,000	190,000
Antiserum, 2.5 units per cc. plus 1:100,000 sulfanilamide	280	130	2,000	1,000	200	90,000	25,000	60,000

These are the only experiments in which the cultures containing sulfanilamide alone did not give lower counts than the control during the period of logarithmic growth and the only experiments in which 2.5 or 12.5 units of antiserum per cubic centimeter prevented an increase in colony count and gave results just as good as those for antiserum plus sulfanilamide. In other words, these results suggest that the serum used in making the cultures must have already contained sulfanilamide.

However, chemical examination of this serum failed to reveal any sulfanilamide, so that some other explanation must be found. It seems probable that the samples of serum from the cord must have contained a substance acting in a manner similar to that of sulfanilamide, but of course there is no way of proving this. Possibly the human serum used in the medium in these experiments was similar to blood F shown in table 1 of the study by Ward,[19] already containing a high titer of substances inimical to the growth of type I pneumococci.

Hartley Broth.—Since the growth curves for the pneumococcus in marrow cultures containing sulfanilamide were similar to the growth curves for the beta hemolytic streptococcus in Hartley broth containing sulfanilamide,[2] and since the combination of antipneumococcus serum plus sulfanilamide in marrow cultures had given results somewhat similar to those obtained with the beta hemolytic streptococcus in marrow culture medium containing human serum, it seemed desirable to study the effects of sulfanilamide plus antipneumococcus serum in Hartley broth.

Such cultures were made in Hartley broth in vaccine vials, the pneumococci being added and mixed in one vial before subdivision was made, just as in the marrow culture experiments. The results are shown in figure 9. The chief differences from the results of experiments with marrow cultures were that the growth curves ascended more steeply and reached a somewhat higher level, apparently because the medium was better adapted for the growth of the organism. There was a greater difference between the highest colony count for the cultures containing sulfanilamide alone and for the control in the broth cultures than for the marrow cultures. The difference between the colony counts for the cultures containing antipneumococcus serum and those for the controls was even more striking than for the marrow cultures with similar initial inoculations and similar concentrations of antiserum. Concentrations as low as 2 units of rabbit antiserum per cubic centimeter produced complete sterility on pour plates made from 1 cc. of the undiluted culture in several cultures with less than 500 pneumococci per cubic centimeter in the initial inoculation. None of the Hartley broth cultures in which horse antiserum was used became sterile, probably because of the prozone phenomenon; but only a few experiments were made, and either a very small dose, 0.03 unit per cubic centimeter, or a very large dose, 50 units per cubic centimeter, was employed.

In all instances in which the cultures did not become sterile too soon for the difference to be detected, a significantly lower count was found

19. Ward, H. K.: Observations on the Phagocytosis of the Pneumococcus by Human Whole Blood: I. The Normal Phagocytic Titre and the Anti-Phagocytic Effect of the Specific Soluble Substance, J. Exper. Med. **51**:675, 1930.

for the cultures containing both antipneumococcus serum and 1 : 10,000 sulfanilamide than for the corresponding cultures containing antiserum alone. As in the marrow culture experiments with the pneumococci, 1 : 100,000 sulfanilamide in combination with antiserum was found much less effective than the 1 : 10,000 concentration. Many more experiments of this type must be made before extensive conclusions are justified, but these results seem sufficiently consistent to warrant their preliminary publication.

COMMENT

These results indicate that in marrow cultures, sulfanilamide alone produces only slight slowing of the rate of multiplication of pneumo-

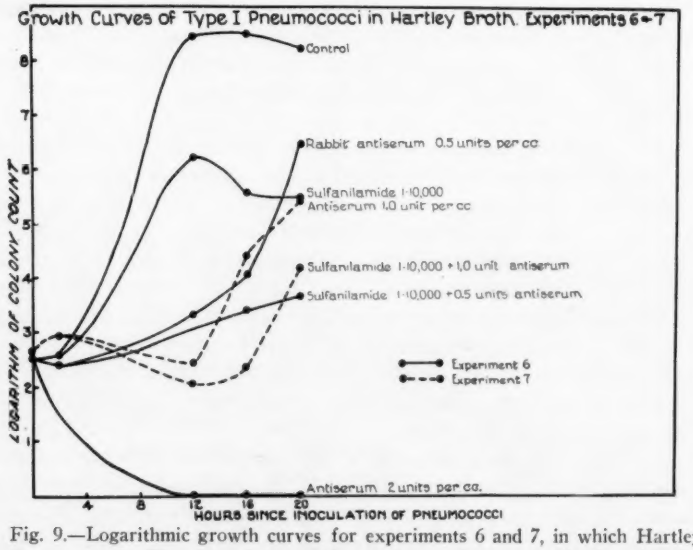

Fig. 9.—Logarithmic growth curves for experiments 6 and 7, in which Hartley broth was used. The data for experiment 6 are shown completely with the exception of the two curves determined for 2 units of rabbit antipneumococcus serum plus 1 : 10,000 and 1 : 100,000 sulfanilamide, which were not significantly different from the curves for 2 units alone. In experiment 7 the curves for the control and for the culture containing sulfanilamide have been omitted, since they almost exactly paralleled the corresponding curves for experiment 6.

cocci, similar to that which has been repeatedly demonstrated with sulfanilamide for other organisms [20] in ordinary bacteriologic mediums.

20. Long, P. H., and Bliss, E. A.: Para-Amino-Benzene-Sulfonamide and Its Derivatives: Experimental and Clinical Observations on Their Use in the Treatment of Beta-Hemolytic Streptococcic Infection; a Preliminary Report, J. A. M. A. **108**:32 (Jan. 2) 1937. Osgood.[2]

This explains why treatment with sulfanilamide only delays the death of animals, such as the mouse, which show a practically 100 per cent mortality for untreated pneumococcic infections. Even small concentrations of antipneumococcus serum are more effective in marrow cultures or in broth than sulfanilamide in 1 : 10,000 concentration, but unless the dose of antiserum is not so great as to produce sterility by itself, the use of sulfanilamide with antiserum is more effective than the use of either alone. The production of sterility in broth cultures of pneumococci indicates that the action of rabbit antipneumococcus serum is not due entirely to its effect on phagocytosis and that the antiserum must contain some bactericidins or bacteriolysins. These results together with those previously published [2] suggest that the important variable in the effectiveness of sulfanilamide in different mediums or animals and against different organisms is the presence or absence of substances bactericidal for that micro-organism in the culture medium or in the body fluids of the particular human being or animal. Just how sulfanilamide renders the micro-organism more susceptible to the action of bactericidal substances is not yet known with certainty, but this work suggests that its effect on the production of toxins or aggressins is more important than its bacteriostatic activity and that it has little direct influence on phagocytosis.

For human beings who have some resistance to pneumococci one might expect from these results that sulfanilamide would somewhat improve the mortality over that obtained without antiserum, but when antiserum is available it is so much superior that there is no clinical justification for using sulfanilamide alone. It is also evident that the larger the dose of sulfanilamide, the better the results, but there are now enough data on the toxicity of sulfanilamide to make it doubtful whether doses equivalent to the 1 to 1.5 Gm. per kilogram used in some animal experiments [21] would be safe for administration to human beings. These results also suggest that sulfanilamide given with the present large doses of antiserum may appreciably lower the mortality or that sulfanilamide given in conjunction with a smaller amount of antiserum than is now used should give an equally low mortality at less expense.

Not enough clinical investigation has been done to furnish any statistical value, but we know of 4 cases of type I lobar pneumonia in which treatment with large doses of antiserum and sulfanilamide was given as a result of these studies. All the patients were afebrile within twenty-four hours and were able to leave the hospital within four to seven days after the onset of the illness. Two patients with type III pneumonia recovered after the administration of large doses of sulf-

21. Rosenthal.[4a] Cooper, Gross and Mellon.[4b] Rosenthal, Bauer and Branham.[4c] Kreidler.[4e] Schmidt.[4f] Bauer and Rosenthal.[4h]

anilamide alone.[22] It is evident that in a large series of cases carefully controlled studies should be made, the use of antiserum alone being alternated with the use of antiserum plus sulfanilamide. Should such studies indicate that the investigations here recorded on experimental infections in human marrow cultures are applicable to clinical infections, this method will have been demonstrated to be a useful adjunct to animal experimentation and clinical investigation.

SUMMARY

Culture of human marrow makes possible a type of control not attainable in animal experimentation or in clinical investigation. In human marrow cultures, sulfanilamide exhibits a slight bacteriostatic action on pneumococcic infections which is increased by an increase in concentration. Even 0.3 unit per cubic centimeter of specific antipneumococcus serum is more effective against the type I pneumococcus than is sulfanilamide alone. Sulfanilamide plus any given dose of antiserum less than the amount which will by itself reduce colony counts to nearly zero is more effective than corresponding doses of antiserum alone. These effects do not depend chiefly on phagocytosis. The results support the view that sulfanilamide renders the organism more vulnerable to bactericidal substances present in the serum. If the results of these in vitro experiments on the interaction of therapeutic and noxious agents in the presence of living human cells are applicable to infections in human beings, sulfanilamide therapy should be of value in pneumococcic pneumonia and might delay death in pneumococcic meningitis, but it will not prove as effective as even small amounts of type-specific antiserum. If used in conjunction with antiserum it should further lower the mortality with the present doses of antiserum or should give an equally low mortality with smaller doses of antiserum. The use of both sulfanilamide and therapy designed to introduce or develop specific bactericidins should be investigated further as a possibly effective treatment for infections which are relatively resistant to the action of sulfanilamide alone.

22. These cases were reported in a personal communication from Dr. J. V. Straumfjord, Astoria, Ore., and Dr. W. C. Hunter, Portland, Ore.

HYPERPARATHYROIDISM DUE TO IDIOPATHIC HYPERTROPHY (HYPERPLASIA ?) OF PARATHYROID TISSUE

FOLLOW-UP REPORT OF SIX CASES

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AND

ESTHER BLOOMBERG, B.S.

BOSTON

In 1934 the first paper on this subject from the clinic of the Massachusetts General Hospital was published.[1] In that report three cases of hyperparathyroidism, from a series of nineteen cases of the condition in which the diagnosis was proved by operation, were cited in which pathologic examination showed what was then termed diffuse hyperplasia of all parathyroid tissue, rather than one or more circumscribed adenomas. It was pointed out that these cases represented a separate disease entity. Since that time the total number of cases of proved hyperparathyroidism at the Massachusetts General Hospital has increased to thirty-five, and the number of cases of "hyperplasia" has increased to six. As far as we are aware, a diagnosis of hyperplasia has not been made during life in other clinics, and these six patients remain a unique group. This paper is a follow-up report of the six cases. Some of the questions which could not be answered at the time of the first publication can now be disposde of; others remain completely obscure.

For those not familiar with the earlier publications it should be emphasized again that the histologic picture of the parathyroid glands in these cases is entirely different from that seen in cases of compensatory hyperplasia of parathyroid tissue, e. g., with long-standing renal insufficiency, rickets, osteomalacia or pregnancy.[2] By "compensatory hyperplasia" is meant the condition encountered in cases in which there

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Read in abstract before the Association of American Physicians, Atlantic City, N. J., May 4, 1937.

From the Medical Service of the Massachusetts General Hospital and the Department of Medicine, Harvard Medical School.

1. Albright, F.; Bloomberg, E.; Castleman, B., and Churchill, E. D.: Hyperparathyroidism Due to Diffuse Hyperplasia of All Parathyroid Glands Rather Than Adenoma of One, Arch. Int. Med. **54**:315 (Sept.) 1934.

2. Albright, F.; Drake, T. G., and Sulkowitch, H. W.: Renal Osteitis Fibrosa Cystica, Bull. Johns Hopkins Hosp. **60**:377, 1937.

is an increased need for the hormone if the calcium level of the serum is to be maintained at a normal value. By "idiopathic hyperplasia" is meant the condition here under discussion, in which some influence is apparently driving the parathyroid tissue to produce more hormone than is required, with resulting hypercalcemia and all the sequelae of primary hyperparathyroidism. The hyperthyroidism of exophthalmic goiter is a somewhat analogous example, wherein a condition other than neoplasia gives rise to more hormone than is needed.

Two interesting questions were brought up in the first paper concerning this disease. The first was academic and still remains a question. It concerns the nature of the influence causing the changes in the parathyroid glands. As previously pointed out, there seemed considerable circumstantial evidence to suggest an overabundance of a pituitary parathyrotropic factor. Some observations have been made which do not substantiate or disprove this possibility. These will be discussed after the clinical data have been given.

The second question was a practical one and one which fortunately can now be answered explicitly. The question arose whether it would be possible to effect a permanent cure by operation. In the earlier paper this aspect of the problem was summed up in the following statement:

> If it is learned by experience that there is a certain fixed amount of hyperplastic tissue which can be left in place—too little to regenerate and again cause hyperparathyroidism, but sufficient to prevent the parathyreoprivic state—then, and only then, will the condition be amenable to surgical treatment.

There was another question embodied in this part of the problem—whether the influence causing the pathologic condition of the parathyroid tissue was a permanent one. The disquieting thought occurred that a patient might have sufficient parathyroid tissue removed to cause a return to the isoparathyroid state, while the remaining tissue stayed abnormal; however, such a patient might find himself in a serious situation if the underlying influence causing the pathologic condition of the parathyroid tissue corrected itself in the course of time.

REPORT OF CASES [3]

CASE 15.[3a]—A more complete history is given in a previous article.[1] In brief, the diagnosis of hyperparathyroidism was first made in November 1933, when the patient, a widow of 62, entered the hospital for the third time with the diagnosis of renal calculi. She had been passing gravel for seven years, so that it is fair

3. The number of the case refers to the series of cases of proved hyperparathyroidism studied at the Massachusetts General Hospital, the same numbering being used in all publications. This particular case was referred to in various articles.

3a. Albright, Bloomberg, Castleman and Churchill.[1] Albright, Aub and Bauer.[8] Churchill and Cope.[9a] Castleman and Mallory.[9c]

to assume that the condition was present at least seven years before her admission to the hospital in 1926. The menopause occurred in 1923. There was no evidence of osseous disease in 1933, which agreed with the finding of an only slightly elevated phosphatase level of the serum. Renal function was only slightly impaired,

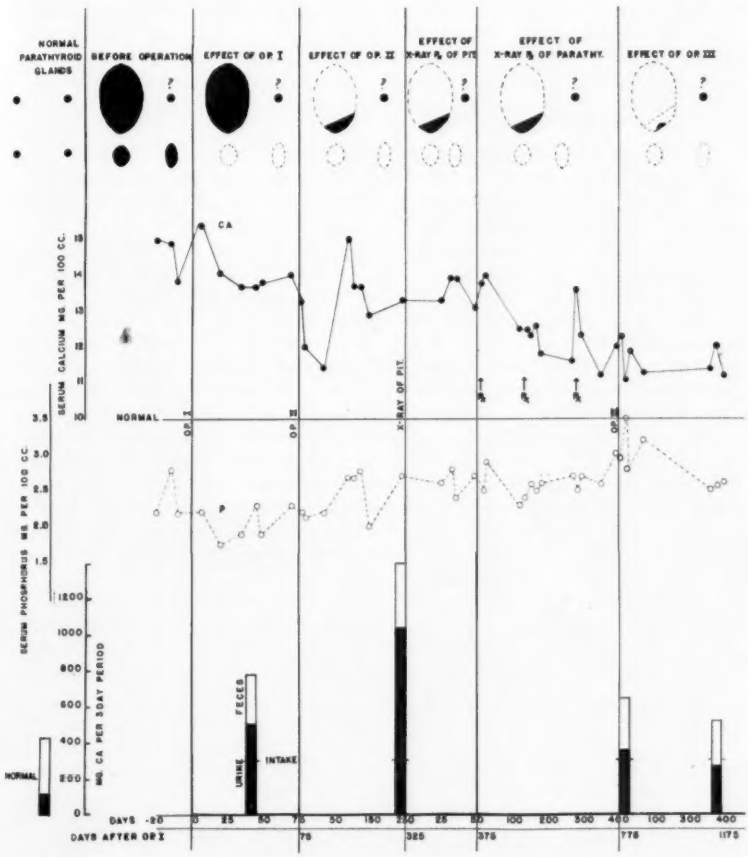

Fig. 1 (case 15).—Diagram showing the effect of various therapeutic procedures on the degree of hyperparathyroidism, as judged by the serum calcium and inorganic phosphorus levels and the excretion of calcium. One gland was never seen and is depicted as a gland of normal size and marked with a question mark. Note that it was not until after the third operation that the condition was brought under control.

and there was no retention of nonprotein nitrogen. She had, in addition, rheumatic heart disease, with mitral stenosis and auricular fibrillation.

In figure 1 the initial calcium and inorganic phosphorus values of the serum are recorded, together with the effect of various therapeutic procedures on these

values. In December 1933 Dr. Edward D. Churchill removed both lower enlarged parathyroid glands. These measured 1.5 by 1.2 by 0.5 cm. and 1.8 by 0.8 by 0.6 cm., respectively, and together weighed 1,100 mg. This procedure had no effect on the degree of hyperparathyroidism, as judged by the serum calcium and phosphorus values and the metabolic data (fig. 1 and table).

At a second operation, in February 1934, Dr. Churchill found a large right upper parathyroid gland (5.0 by 3.0 by 1.3 cm.) weighing 10,000 mg. but could not find the left upper parathyroid gland. He resected the gland, leaving behind a piece with an estimated weight of 500 mg. (four normal parathyroid glands weigh about 150 mg.). This second procedure was followed by only transient improvement of the blood values.

In July 1934 it was decided to try large doses of estrogenic substance. The thought behind this was as follows: As pointed out in the previous paper, there was considerable indirect evidence that the underlying disorder might be an excess of a pituitary parathyrotropic hormone. This patient's condition came on at about the time of the menopause, when there starts an increased functioning of the follicle-stimulating hormone of the anterior lobe of the pituitary body. It seemed possible that the hypothetic parathyrotropic hormone might be either identical with the follicle-stimulating hormone or, what was more probable, influenced by the same factors. Several of the pituitary hormones are decreased by treatment with estrogenic substance: follicle-stimulating hormone,[4] diabetogenic hormone[5] and growth hormone.[6] Hence 600 rat units of estradiol[7] was given three times daily for twenty-three days. The serum calcium and phosphorus values remained unaltered (fig. 1). After cessation of this treatment there was vaginal bleeding, as was to be expected from such large doses of estrogenic substance. In the light of more recent studies we believe that the amount of treatment may have been too little to return the follicle-stimulating hormone level to normal. Some data with larger doses of estrogen in the form of estradiol benzoate were obtained at a much later date which rather indicated that the serum calcium level was decreased with this therapy (table).

In accordance with the same line of reasoning irradiation of the pituitary gland was given early in December 1934. The patient received 800 roentgens to each side of the skull, focused on the pituitary body. This procedure was again without effect on the abnormal calcium and phosphorus values of the serum (fig. 1).

In January 1935 it was decided to try irradiation over the parathyroid tissue left in place at the second operation. Reports have appeared in the literature of cases in which supposed hyperparathyroidism has been "cured" by irradiation of the cervical region. Experience from this clinic has made it clear that such

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4. Albright, F., and Halsted, J. A.: Studies on Ovarian Dysfunction: II. The Application of the "Hormonal Measuring Sticks" to the Sorting Out and to the Treatment of the Various Types of Amenorrhoea, New England J. Med. **212**: 250 (Feb. 7) 1935.

5. Barnes, B. O.; Regan, J. F., and Nelson, W. O.: Improvement in Experimental Diabetes Following the Administration of Amniotin, J. A. M. A. **101**:926 (Sept. 16) 1933.

6. Zondek, B.: The Inhibitory Effect of Follicular Hormone on the Anterior Lobe of the Pituitary Gland, Lancet **1**:10 (Jan. 4) 1936.

7. The estradiol used in these investigations was supplied by Dr. Gregory Stragnell and Dr. Erwin Schwenk, of the Schering Corporation, Bloomfield, N. J.

Metabolic Data in Case 15

	Date of Periods (Inclusive)	Period	Calcium, Gm. per Day				Phosphorus, Gm. per Day				Serum Calcium, Mg. per 100 Cc.	Serum Phosphorus, Mg. per 100 Cc.	Rat Units of Estradiol Benzoate per Period
			Urine	Feces	Intake	Balance	Urine	Feces	Intake	Balance			
	1934												
After first operation and before second operation	Jan. 14 to 16	1	0.18	0.09	0.10	−0.17	0.34	0.07	0.46	+0.05			
	Jan. 17 to 19	2	0.17	0.14	0.10	−0.21	0.27	0.17	0.46	+0.02			
	Jan. 20 to 22	3	0.17	0.09	0.10	−0.16	0.29	0.10	0.46	+0.07	13.7	1.9	
	1934												
After second operation	Nov. 23 to 25	1	0.35	0.14	0.10	−0.39	0.61	0.20	0.50	−0.22	13.3	2.7	
	Nov. 26 to 28	2	0.35	0.10	0.10	−0.35	0.50	0.25	0.59	−0.16			
	1936												
Directly after third operation; effect of ingestion of calcium gluconate	Mar. 16 to 18	1	0.13	0.05	0.10	−0.08	0.44	0.23	0.59	−0.08	12.4	3.0	
	Mar. 19 to 21	2	0.12	0.09	0.10	−0.11	0.45	0.22	0.59	−0.08			
	Mar. 22 to 24	3	0.11	0.15	0.10	−0.16	0.33	0.17	0.59	+0.09			
	Mar. 25 to 27	4	0.13	0.16	0.47	+0.18	0.30	0.17	0.59	+0.12	11.1	3.5	
	Mar. 28 to 30	5	0.13	0.21	0.47	+0.13	0.32	0.20	0.59	+0.07			
	Mar. 31 to Apr. 2	6	0.16	0.23	0.47	+0.08	0.37	0.22	0.59	+0.00	11.9	2.8	
	1937												
Final observation and effect of estrogenic substance	Mar. 13 to 15	1	0.10	0.08	0.10	−0.08	0.37	0.17	0.59	+0.05			
	Mar. 16 to 18	2	0.10	0.10	0.10	−0.10	0.35	0.20	0.59	+0.04	11.7	2.6	
	Mar. 19 to 21	3	0.07	0.08	0.10	−0.05	0.38	0.16	0.59	+0.05	12.3	2.5	
	Mar. 22 to 24*	4	0.09	0.08	0.10	−0.07	0.36	0.21	0.59	+0.02	....	...	20,000
	April 1 to 3	5	0.07	0.10	0.10	−0.07	0.34	0.25	0.59	+0.00	....	...	10,000
	April 4 to 6	6	0.07	0.09	0.10	−0.06	0.35	0.26	0.59	−0.02	10.5	2.3	10,000
	April 7 to 9	7	0.07	0.04	0.10	−0.01	0.32	0.11	0.59	+0.16	10.4	2.2	10,000

* Metabolic studies were discontinued for one week as the patient contracted a cold.

treatment is ineffective in cases of parathyroid adenoma.[8] It seemed possible, however, that it might be effective in cases of so-called parathyroid hyperplasia. The patient had her first treatments on January 17 and 19. At this time she received 400 roentgens. The treatment had to be interrupted, however, because of an attack of acute cholelithiasis. On January 20 Dr. Joe V. Meigs performed cholecystostomy and removed about twenty stones from the gallbladder. (Incidentally, these stones contained almost no ash, so that it is unlikely that the hyperparathyroidism had anything to do with them, as, for instance, by increasing the calcium content of the bile.) The patient made an uneventful recovery. It should be noted, however, that the presence of mild diabetes first became apparent at this time. The course of irradiation was completed in May and June, when she received an additional 800 roentgens to the remainder of the right upper parathyroid gland. There was no immediate change in the serum calcium and phosphorus values. She returned in October and received further irradiation. This time it was given on both sides of the neck, on the basis that a fourth parathyroid tumor might have been overlooked. She received 900 roentgens over each of two fields measuring 10 by 10 cm. on either side of the neck. This treatment gave her a sore throat, but the hyperparathyroidism remained the same.

Finally, since it was realized from observations in other cases that too much tissue had been left behind at the second operation, even on the assumption that there was no fourth gland, a third operation was performed by Dr. Churchill in March 1936, twenty-nine months after the first operation and ten years at least after the onset of the illness. The remainder of the right upper parathyroid gland was apparently of the same size as at the time of the second operation. It was resected, an amount of tissue being left in place that was about equivalent to that in a good-sized normal parathyroid gland, measuring 0.6 by 0.3 by 0.9 cm. (fig. 1). Starting two weeks after this operation, the serum calcium value gradually dropped almost to normal (fig. 1). The histologic appearance of the tissue removed was exactly similar to that of the tissue removed at the first two operations (fig. 2). This indicated that the underlying pathologic condition had persisted and also that irradiation in the doses given had had no effect on the tissue. The tissue removed at the third operation weighed only 170 mg. Therefore the estimated value of 500 mg. for the tissue left after the second operation, though the same estimation was made at both the second and the third operation, was considerably too high.

In the accompanying table the calcium and phosphorus values obtained when the patient was receiving a neutral ash, low calcium diet at various stages in the course of the illness are recorded. The calcium values are also recorded graphically in figure 1.

During the two and a half years following the first operation the patient was kept on a fairly low calcium diet to avoid if possible the development of further renal stones. During this period there gradually developed slight roentgenographic evidence of osseous disease but no symptoms thereof. The renal condition remained essentially unchanged. The patient has continued to have slow auricular fibrillation.

CASE 16.[3a]—For the more complete early history reference should be made to the first report.[1] Hyperparathyroidism was discovered in October 1933, when the patient entered the hospital at the age of 26 because of renal colic. There was no

8. Albright, F.; Aub, J. C., and Bauer, W.: Hyperparathyroidism: A Common and Polymorphic Condition as Illustrated by Seventeen Proved Cases from One Clinic, J. A. M. A. **102**:1276 (April 21) 1934.

evidence of osseous disease, the serum phosphatase level was normal and there was no impairment of renal function. If one judges from the duration of the polyuria, the condition had been present for one year or slightly longer.

The preoperative serum calcium and phosphorus values, together with later values, are shown in figure 3. Dr. Churchill performed the first operation on Feb. 16, 1934, and removed from the right side of the neck two enlarged glands,

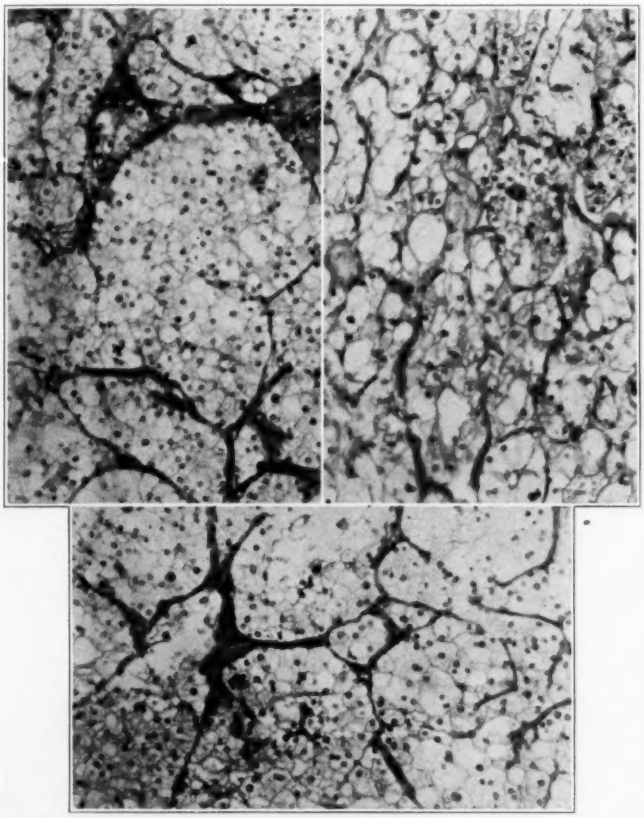

Fig. 2 (case 15).—Photomicrograph of parathyroid tissue removed at three operations, twenty-nine months having elapsed between the first and the third operation. Note that all three specimens are similar.

measuring 4.5 by 3.5 by 2.5 cm. and 1.5 by 1.0 by 0.6 cm., respectively, and together weighing 15,600 mg. The other side of the neck was not explored. This was before the true nature of the disease was appreciated and before it was realized that two additional enlarged glands were to be expected on the other side. The histologic sections showed hyperparathyroidism with so-called diffuse hyperplasia. In spite of the incompleteness of the operation, the serum calcium value imme-

diately fell to normal, and the urinary calcium and phosphorus values immediately dropped (fig. 3). The metabolic changes will be discussed in another paper.

Fifteen months after the operation the serum calcium level was still normal. However, twenty-six months after operation it was clear that hyperparathyroidism was again present (fig. 3). In June 1936 Dr. Oliver Cope explored the other side of the neck and found, as expected, two enlarged glands. The upper gland,

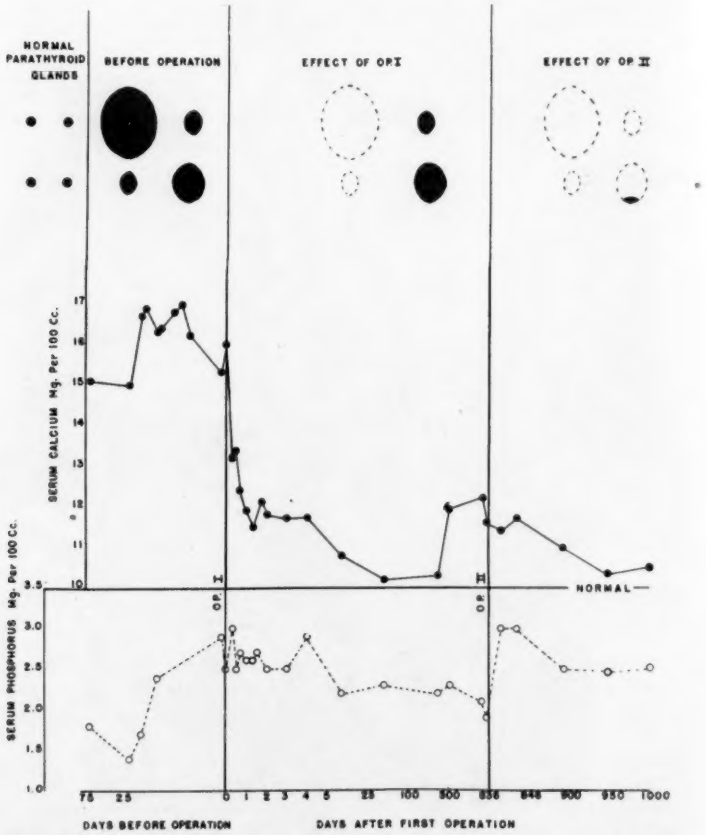

Fig. 3 (case 16).—Diagram showing the serum calcium and inorganic phosphorus levels in relation to two operations on the parathyroid glands.

measuring 1.1 by 0.8 by 0.4 cm. and weighing 455 mg., was excised in toto. The lower gland was resected, a piece estimated as weighing 125 mg. being left behind. The part removed measured 1.5 by 1.0 by 0.8 cm. and weighed 3,350 mg. The histologic picture was exactly the same as that of the tissue removed at the first operation. After this procedure the serum calcium value slowly returned to normal (fig. 3).

CASE 17.[3a]—A more complete history was given in a previous publication.[1] The diagnosis of hyperparathyroidism was made in December 1933, when the patient, a married woman of 55, entered the hospital with a fourteen months' history of renal lithiasis. The menopause occurred six years before her admission to the hospital. There was no skeletal involvement or renal impairment.

In figure 4 the preoperative serum calcium and phosphorus values are shown, together with subsequent values. On Feb. 14, 1934, Dr. Churchill explored the

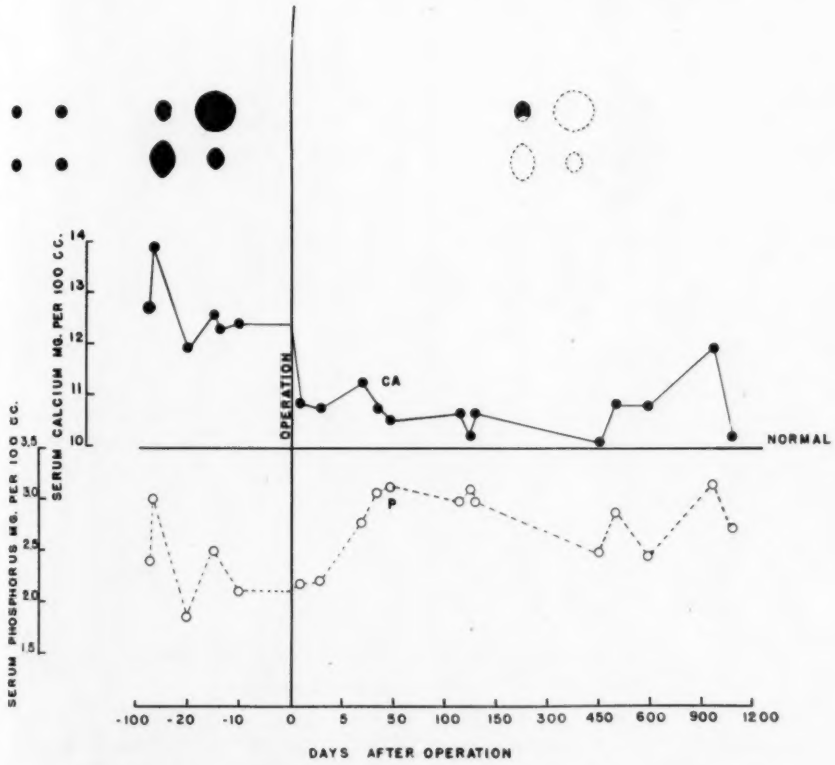

Fig. 4 (case 17).—Diagram showing the serum calcium and inorganic phosphorus levels in relation to an operation on the parathyroid glands. At the top of the diagram the parathyroid glands before and after operation are schematically indicated and compared with four normal parathyroid glands at the extreme left.

parathyroid region and found four hyperplastic glands. Three of these were removed, and a specimen was taken from the fourth gland for biopsy. The respective weights and dimensions of the removed glands were: left upper, 2,000 mg. and 2.0 by 2.0 by 1.0 cm.; left lower, 600 mg. and 1.0 by 0.8 by 0.4 cm.; right lower, 800 mg. and 1.8 by 1.2 by 0.4 cm. The right upper gland, which was

left in place, unfortunately was not measured but was about the size of either of the two lower parathyroid glands. Roughly, about 500 mg. of tissue was left behind. After the operation the serum calcium level immediately fell to normal (fig. 4). The values were still satisfactory three years later.

CASE 23.[9]—For the detailed history, reference should be made to previous publications.[10] This patient, a married man aged 41, a telephone lineman, entered the hospital in February 1934. His symptoms at that time were due to what proved

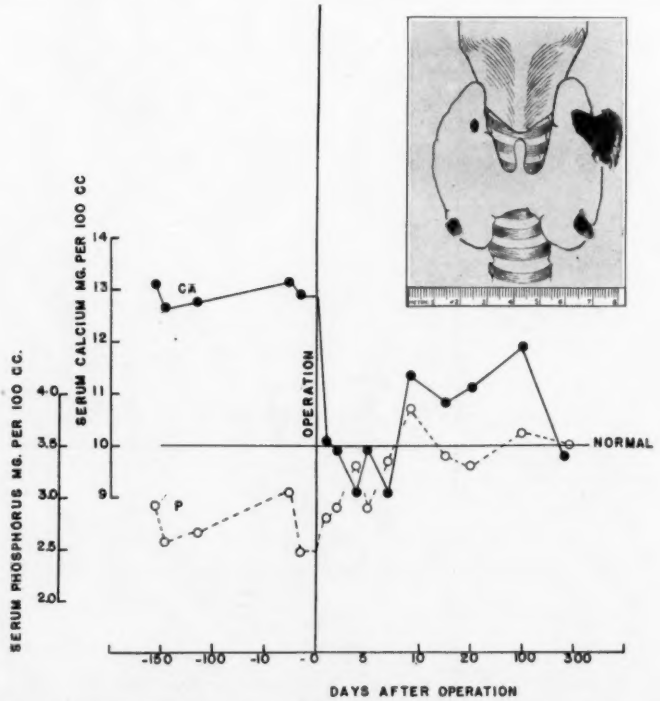

Fig. 5 (case 23).—Diagram showing the effect of an operation on the serum calcium and inorganic phosphorus levels. The insert shows the three glands that were removed photographed with a conventionalized drawing as a background. The scale is in centimeters. The right upper parathyroid gland was not removed, and its size is indicated by an elliptic black area.

9. (*a*) Churchill, E. D., and Cope, O.: The Surgical Treatment of Hyperparathyroidism, Based on Thirty Cases Confirmed by Operation, Ann. Surg. **104**:9, 1936. (*b*) Albright, Aub and Bauer.[8] (*c*) Castleman, B., and Mallory, T. B.: Pathology of the Parathyroid Glands in Hyperparathyroidism, Am. J. Path. **11**:1, 1935. (*d*) Albright, F.: Hyperparathyroidism: A Case with Several Unusual Features, Including a Probably Non-Related Chondrosarcoma, Bence-Jones Proteinuria, and Hyperplasia of All Parathyroid Tissue, M. Clin. North America **18**:1109, 1935.

10. Churchill and Cope.[9a] Albright.[9d]

to be chondrosarcoma of the left ilium. It is doubtful that this was in any way related to the hyperparathyroidism. Six months previously he had had renal colic on the left side, and some stones had been removed from the bladder.

Roentgen examination showed prostatic calculi. Renal function tests gave excellent results. The roentgenograms of the bones, aside from those of the left ilium, were not remarkable; the serum phosphatase level was only slightly elevated. Although the diagnosis of hyperparathyroidism was made at the time of his first admission to the hospital, the chondrosarcoma was removed at that time, and it was not until July 12 that Dr. Churchill explored the parathyroid region.

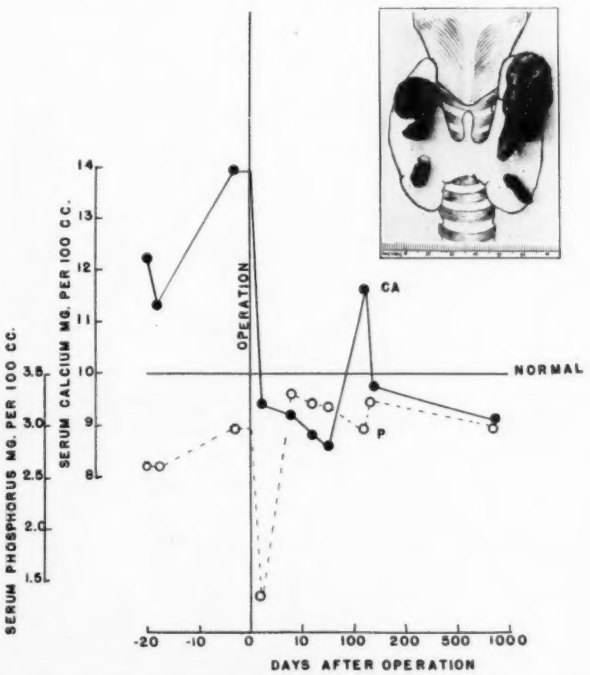

Fig. 6 (case 25).—Diagram showing the effect of an operation on the serum calcium and inorganic phosphorus levels. The insert shows a photograph of the parathyroid tissue removed. The area enclosed by a dotted line indicates the amount of the right lower parathyroid gland left in place.

The preoperative serum calcium and phosphorus values are shown in figure 5, together with subsequent values. At operation all four parathyroid glands showed so-called hyperplasia. The right upper one was not enlarged, measuring 0.7 by 0.4 cm. It was not disturbed, except that a small specimen was removed for biopsy. The three remaining glands were removed. They had the following measurements and weights: right lower, 130 mg. and 0.8 by 0.6 by 0.3 cm.; left upper, 2,180 mg. and 3.0 by 1.7 by 0.8 cm.; left lower, 160 mg. and 1.1 by 0.6 by 0.3 cm. The blood values returned promptly to normal and have remained satisfactory ever since (fig. 5).

CASE 25.[11]—For the more complete history, reference should be made to a previous report.[9a] The patient entered the hospital in October 1934, at the age of 39. There was a four months' history of renal colic on the right side. Roentgenologic examination revealed a stone in the right ureter and no evidence of osseous disease. The serum phosphatase level was normal. Renal function tests showed normal excretion. The stone was removed.

The first serum calcium and phosphorus values are shown in figure 6, together with later values. Dr. Cope explored the parathyroid region on October 27 and

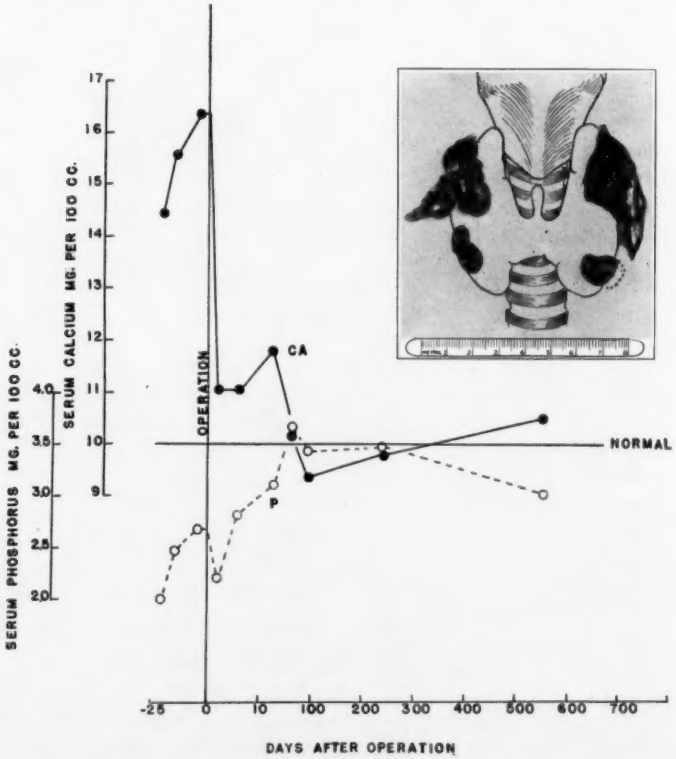

Fig. 7 (case 26).—Diagram showing the effect of operation on the serum calcium and inorganic phosphorus levels. The insert shows a photograph of the parathyroid tissue removed. The area enclosed by a dotted line represents the tissue left in place.

11. Albright, F.; Sulkowitch, H. W., and Bloomberg, E.: Further Experience in the Diagnosis of Hyperparathyroidism, Including a Discussion of Cases with a Minimal Degree of Hyperparathyroidism, Am. J. M. Sc. **193**:800, 1937. Churchill and Cope.[9a] Castleman and Mallory.[9c]

found all four parathyroid glands enormously enlarged. He removed all the parathyroid tissue except a piece of the lower right gland. This piece measured 0.7 by 0.4 by 0.35 cm. and was estimated to weigh 40 mg. The weights of the removed glands were: left upper, 4,960 mg.; right upper, 1,630 mg.; left lower, 110 mg.; right lower (subtotal), 100 mg. After the operation the serum calcium value returned to normal and has remained satisfactory ever since (fig. 6).

CASE 26.—For a more complete history, reference should be made to a previous report.[9a] The patient, a housewife aged 57, was admitted in January 1935. The right kidney was removed elsewhere, in 1931, because of nephrolithiasis. During the six months prior to the present admission to the hospital she had vague aches and pains throughout the skeleton. Roentgenographic studies showed generalized decalcification, indicating the diagnosis. Biopsy of bone showed osteitis fibrosa. Renal function tests showed normal functioning of the remaining kidney.

The preoperative serum calcium and phosphorus values are shown in figure 7, together with later values. In spite of definite skeletal involvement, the serum phosphatase value was not elevated. On February 9 Dr. Cope explored the parathyroid region and found four enormously enlarged glands. He removed all except a piece of the left inferior gland, estimated to weigh 225 mg. The weights of the glands removed were as follows: left upper, 6,000 mg.; right upper, 3,750 mg.; left lower (subtotal), 860 mg., and right lower, 590 mg. The combined weight was 11,200 mg. The serum calcium level returned to normal, where it has remained; the patient has improved markedly in weight and strength, and the skeletal pains have disappeared.

COMMENT

A. Nature of the Pathologic Condition of the Parathyroid Tissue.—The question has not been definitely settled whether the enlargement of the glands in this disease is due to hyperplasia and hypertrophy or just to hypertrophy of the cells. The weight of the actual mass of tissue in our six cases ranged from 19,100 to 2,510 mg., roughly one hundred to thirty times normal. Gilmour and Martin[12] found that the mean and standard deviations for the weight of parathyroid tissue are 117.6 ± 4 mg. and 45.97 mg., respectively, for men and 131.3 ± 5.8 mg. and 45.02 mg. for women. The diameters of the individual cells are approximately three to four times normal size. Castleman and Mallory[9c] gave as the diameter of the normal chief cell 6 to 8 microns and as the diameter of the cells in question 10 to 40 microns. Since the volume of a sphere increases as the cube of the radius, a fourfold increase in the radius would cause the volume to increase by sixty-four times. Thus, a large part, if not all, of the increase in size can be explained by hypertrophy of the cells. Further evidence that hyperplasia may be playing a subordinate role is the fact that the histologic appearance is entirely different from that seen in conditions of known hyperplasia, e. g., rickets[12a] and renal insufficiency.[2]

12. Gilmour, J. R., and Martin, W. J.: The Weight of the Parathyroid Glands, J. Path. & Bact. **44**:431, 1937.

12a. Albright, F.; Butler, A. M., and Bloomberg, E.: Rickets Resistant to Vitamin D Therapy, Am. J. Dis. Child. **54**:529 (Sept.) 1937.

This hypothesis should be subject to proof by careful measurement of cell diameters. The problem is complicated by the fact that there is considerable variation in the size of cells (possibly due to fixing) in different sections of the same specimen. The subject is under investigation by Dr. Castleman.

One receives the impression from studying sections of tissue from these six patients that the parathyroid disturbance is an "all or none" disturbance, in that there is either a maximum degree of disturbance or none. Thus, the cells in case 23, in which there was the smallest mass of tissue, were not noticeably smaller than those in cases 16 and 26, in which there were the largest masses. No mild degree of the disease, with only slight enlargement of the cells, has been encountered. It has not been disproved as yet that the difference between case 23, in which there was 2,510 mg. of tissue, and case 26, in which there was 11,425 mg. of tissue, may not have been due to the amount of tissue which the patients had before the disease started.

Such a conception fits in with the fact that all three operations in case 15 (fig. 2) showed the same histologic changes in the parathyroid tissue and that the piece left in at the second operation apparently was just the same when seen again twenty-five months later, at the third operation. The initial cure in case 16 after the first operation, followed by a later relapse, however, suggests that the two remaining glands had increased in size between the first and the second operation. However, it is perhaps significant that this was the only one of the six cases in which at any time there was shown a tendency for the condition to grow worse as regards the degree of hyperparathyroidism, and in this case the facts are not conclusive.

The data in case 15 make it clear that the condition once it is established remains indefinitely. Thus the history shows that the condition was present in 1926; it was still present at the third operation, in 1936. Therefore it is fair to assume that the underlying abnormality was chronic. This, as previously discussed, is an important point with regard to a decision as to how much tissue to remove at operation, because once the patient has had the parathyroid glands resected to a point desirable for the so-called hyperplastic state, it would be most unfortunate to have the state correct itself.

In figure 8 the serum calcium values have been charted against the weights of parathyroid tissue at the various stages in the treatment in these six cases. The pathologic picture of the tissue being absolutely uniform, one would expect a definite correlation between the amount of tissue and the amount of serum calcium. Such was the case, especially if one believes that the two values recorded in case 15 which were not in line deviated because of a fourth parathyroid gland, which was not found. No such correlation occurs in cases of parathyroid adenoma.

The foregoing observations suggest that the condition under discussion has not yet been proved to be neoplasia or hyperplasia but may be a disorder of function. It seems possible, for example, that under normal conditions the parathyroid hormone is made and released according to the needs of the body, the stimulus perhaps being a tendency for the serum calcium content to fall below 10 mg. per hundred cubic centimeters. In the condition under discussion there may be a disturbance in the production of parathyroid hormone by the cells, the production being tremendously speeded up. The release mechanism

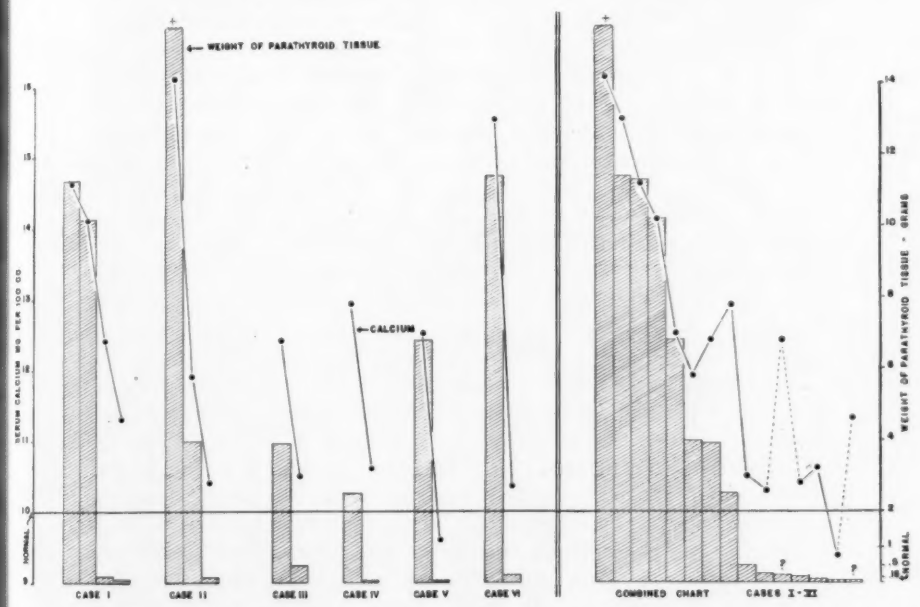

Fig. 8.—Diagram showing the correlation between the weight of the parathyroid tissue at the various stages in the treatment of the six patients and the level of the serum calcium. Question marks and dotted lines indicate points where there may be a marked error in the diagram because the fourth gland was never found in case 15. Cases I to VI, inclusive, in the diagram correspond to cases 15, 16, 17, 23, 25 and 26, respectively, described in the text.

may still attempt, with only partial success, to hold the hormone back as much as possible, and the swelling of the glands may be due to the fact that the cells are distended with hormone. It would be helpful if one knew whether such tissue contains more hormone per unit of weight than other parathyroid tissue. It would also be interesting to know whether the swollen aspect of the cells disappears when the amount

of tissue has been reduced sufficiently so that the serum calcium value is on the low side (case 26). These wholly theoretic observations are included here in the hope that the next person encountering such a patient will save some tissue for biologic assay.[13]

Case 23 is of interest in that one gland, although histologically similar to the others, was no larger than a normal gland—weighing about 40 mg. The largest gland in this case weighed 2,180 mg. If one assumes that this gland weighed one-fortieth as much, or 54.5 mg., before the disease occurred, then the small gland must have weighed 1 mg. This probably explains why normally one often can find only three glands, although embryologic studies have shown that four glands are almost sure to be present.[14]

B. Treatment of the Disease.—Regardless of what the exact nature of the disease is, it can now be said with a fair degree of assurance that the condition is amenable to surgical treatment. As previously discussed, the underlying pathologic condition is persistent (for at least ten years in case 15), and if the surgeon removes all the tissue except about 200 mg., it appears from the data assembled that there should be a permanent cure. In all the cases here considered the patient has been followed for over two years postoperatively, and one patient (case 17) who had about 400 mg. of tissue left in place is still in an isoparathyroid state, over three years after operation.

It should be noted that if one leaves out of consideration case 15, in which the possibility of a fourth gland is not unlikely, the removal of all but 400 mg. or less of parathyroid tissue was attended by cure. There are no data for remnants weighing between 400 and 2,510 mg. (before operation in case 23). Therefore, one cannot say that the leaving of 1,000 mg., for example, might not be attended by permanent cure. This is an important question, as the more that can be left in without causing recurrence, the safer the operation will be and the less a subsidence of the so-called hyperplastic state is to be feared. It should be remembered, of course, that the amount of parathyroid tissue which must be left in place is increased if renal insufficiency [15] or marked osseous disease is present.[9a]

C. Cause of the Pathologic State of the Parathyroid Glands.—The cause of the pathologic condition remains completely obscure. In the previous paper considerable circumstantial evidence was presented suggesting that the condition may be secondary to an excess of pituitary

13. Dr. Collip has informed us that the tissue should be preserved in ten parts of acetone for this procedure.

14. Norris, E. H.: The Parathyroid Glands and the Lateral Thyroid in Man: Their Morphogenesis, Histogenesis, Topographic Anatomy and Prenatal Growth, Publication 479, Carnegie Institution of Washington, 1937, p. 247.

15. Albright, Drake and Sulkowitch.[2] Churchill and Cope.[9a]

parathyrotropic substance. No confirmatory evidence has been obtained. It should be noted that the changes in rabbit parathyroid glands described by Hertz and Kranes as the result of injections of pituitary substance were of the nature of true hyperplasia, with mitotic figures. Mitoses have not been noted in any of the parathyroid tissue in the disease under consideration.

Roentgenographic examinations of the skulls in all six cases failed to demonstrate any evidence of a pathologic condition of the pituitary body. Roentgen radiation of the pituitary body was without benefit in case 15. Massive treatment with estrogenic substance (which depresses the pituitary hormone in many cases) had no effect in case 15. Whereas two of the patients (cases 15 and 17) had passed the menopause and had the expected increase of follicle-stimulating hormone in the urine, no such increase was found in cases 16, 23 and 25. In cases 15 and 26 the sugar curves were definitely typical of diabetes, but there was no suggestion of diabetes in the other cases, and the sugar curve was perfectly normal in case 16.

SUMMARY AND CONCLUSIONS

Six cases of hyperparathyroidism with what has previously been termed primary hyperplasia of the parathyroid glands are reviewed.

It is pointed out that the pathologic condition of the parathyroid glands is histologically dissimilar from that in cases of undoubted hyperplasia of parathyroid tissue, that it has not yet been shown that the enormous enlargement of the glands in this condition (about thirty to one hundred times) cannot be explained by hypertrophy of the cells and that the condition may be a disorder of hormone production rather than hyperplasia.

The studies showed that all the glands from all six patients on all occasions revealed a similar histologic picture; it is suggested that the tissue change is an "all or none" one.

A distinct correlation was observed between the weight of the parathyroid tissue and the degree of hyperparathyroidism; this was in marked contrast to the situation in cases of parathyroid adenoma.

Evidence is presented that the underlying cause of the changes in the parathyroid glands is a chronic one (in one case the condition had existed for at least ten years).

There was little, if any, evidence of a tendency for the parathyroid tissue left in place after partial resection to regenerate, so the condition is apparently amenable to permanent surgical cure. The optimum amount of tissue to be left in place at operation has not yet been determined, but anything less than 400 mg. is probably not too much.

No evidence has been obtained to confirm the hypothesis that the condition is secondary to overactivity of some pituitary hormone.

REMOVAL OF INTRAVENOUSLY INJECTED BROMSULPHALEIN FROM THE BLOOD STREAM OF THE DOG

A COMPARISON OF THE REMOVAL OF INTRAVENOUSLY INJECTED BILIRUBIN AND THAT OF BROMSULPHALEIN

MOORE A. MILLS, PH.D.*
AND
CARL A. DRAGSTEDT, M.D., PH.D.
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We [1] have previously reported observations on the effect of various experimental procedures on the removal of intravenously injected bilirubin from the blood stream of the dog. These observations may be briefly summarized as follows: The normal rate of removal is extremely rapid when compared with that in man; it is characterized by a prompt disappearance of 50 to 60 per cent of the amount injected within the first five minutes and a subsequent progressive decline in the plasma levels so that bilirubin is no longer detectable in the plasma after sixty to ninety minutes with a 5 mg. per kilogram dose and after ninety to one hundred and twenty minutes with a 10 mg. per kilogram dose; anesthesia produces a definite impairment in the rate of removal; obstruction of the bile ducts results in an immediate complete impairment of removal; reticuloendothelial blockade produces a slight but definite impairment, and the intravenous administration of decholin sodium has only minor and perhaps negligible effects. The removal of intravenously injected dyes such as bromsulphalein from the blood stream has been used as a method of studying hepatic function. However, there are suggestions in the literature that the removal of bromsulphalein from the blood stream might not be affected by some of the aforementioned procedures to the same degree as the removal of bilirubin. For example, various workers, including ourselves, have found that reticuloendothelial blockade produces a marked impairment in the removal of bromsulphalein from the blood stream.[2] Also it is indicated by the work of Snell,

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1. Dragstedt, C. A., and Mills, M. A.: Am. J. Physiol. **119**:713, 1937.

2. (*a*) Herlitz, C.: Acta pædiat. (supp. 5) **12**:1, 1931. (*b*) Klein, R., and Levinson, S. A.: Proc. Soc. Exper. Biol. & Med. **31**:179, 1933. (*c*) Mills, M. A., and Dragstedt, C. A.: ibid. **34**:228, 1936.

Greene and Rowntree [3] and others that obstruction of the bile ducts is not followed by immediate impairment of dye removal, as is the case with bilirubin. The present study was therefore designed to determine the effects of various experimental procedures on the rate of removal of bromsulphalein so that a comparison with the observations on bilirubin could be made, in the hope that the data would aid in the understanding of the interrelations between the Kupffer cells and the parenchymal cells of the liver and thus in the interpretation of various tests of hepatic function.

METHODS

The dose of bromsulphalein employed in the tests was 2 mg. per kilogram of body weight injected intravenously. Samples of blood (oxalated) were drawn five and thirty minutes after the injection. The plasma was alkalized by the addition of 2 drops of tenth-normal sodium hydroxide, and readings were made in a comparator box against freshly prepared standards. As is customary with this technic, the reported values represent the percentage of dye present in the plasma and are computed on the assumption that the volume of blood is 10 per cent of the body weight.

In the course of our studies of the plasma levels of bilirubin and bromsulphalein after intravenous injection there have been definite indications that the rates of decline of values for these substances have a biphasic character. The first, or primary, phase may be described as that obtaining during the first five minutes (roughly) and the second, or secondary, phase as that subsequent to this five minute interval. The evidence presented in this paper indicates that various procedures may primarily affect one or the other phase, so that it seems of some advantage to use these terms descriptively in relating our observations.

RESULTS

1. *Removal of Bromsulphalein in Unanesthetized Dogs.*—The data for 18 normal dogs are shown in table 1. The values agree in general with those obtained by others. Bromsulphalein disappears from the blood with great rapidity; 85 to 95 per cent is removed within five minutes and the remainder within thirty minutes. The rate of removal is thus greater than that of bilirubin, 50 to 60 per cent of which disappears within five minutes, sixty to ninety minutes being required for complete removal of the remainder. This ratio holds true when amounts of bromsulphalein up to 5 mg. per kilogram are employed. Thus the major part of the injected bromsulphalein leaves the blood stream during the primary phase, while approximately equal amounts of bilirubin are removed during the primary and the secondary phase.

2. *Removal of Bromsulphalein in Dogs During Anesthesia.*—The data for 29 animals are shown in table 1. Surgical anesthesia with ether or ether and barbital of from one to two hours' duration has no

3. Snell, A. M.; Greene, C. H., and Rowntree, L. G.: Diseases of the Liver: Comparative Study of Certain Tests for Hepatic Function in Experimental Obstructive Jaundice, Arch. Int. Med. **36**:273 (Aug.) 1925.

appreciable effect on the rate of removal of bromsulphalein from the blood stream. Comparable degrees of anesthesia materially impair the removal of bilirubin. Chloroform anesthesia or deep or prolonged anesthesia with ether and barbital affects the rate of removal of bromsulphalein (see also the article by Rosenthal and Bourne [4]).

3. *Removal of Bromsulphalein in Dogs After Ligation of the Bile Ducts.*—The data for 7 animals after various types of ligation and after various intervals are shown in table 1. Even after ligation of both the common and the cystic duct there is no appreciable reduction in the rate of removal of bromsulphalein for periods up to three hours. Our results agree in general with those of Snell, Greene and Rowntree,[3] who employed phenoltetrachlorphthalein after various types of ligation in dogs and reported that no retention of dye occurred for a number

TABLE 1.—*Rate of Removal of Intravenously Injected Bromsulphalein from the Blood Stream*

Procedure	Number of Dogs	Bromsulphalein, Mg. per Kg.	Bromsulphalein, % 5 Min.	30 Min.	Conditions
Normal animals...	3	2	5	0	Normal
	10	2	10	0	Normal
	3	2	15	0	Normal
	2	5	15	0	Normal
Anesthesia.....	3	2	10	0	Ether
	12	2	5-15	0	Ether-barbital
	4	2	10	0	Avertin
	5	2	15-50	0-15	Chloroform
Reticuloendothelial blockade	14	2	30-80	10-50	Complete data given previously [2c]
Ligation of ducts	2	2	5-20	0	Common duct, 1 hr.
	2	2	15-35	0-20	Common duct, 24 hr.
	1	2	10	0	Common and cystic ducts, 1 hr.
	1	2	15	10	Common and cystic ducts, 3 hr.
	1	2	10	10	Common and cystic ducts, 4 hr.
Decholin.....	2	5	45-50	10-15	5 cc. decholin 5 min. before test
	2	2	40-60	0-5	2.5 cc. decholin 5 min. before test

of hours or until bilirubinemia resulting from the obstruction was present. Our findings obtained with bromsulphalein are in striking contrast to those obtained with bilirubin. Even if the bilirubin is injected as soon after ligation of the ducts as is practicable, the secondary phase of removal of bilirubin is completely inhibited.

4. *Removal of Bromsulphalein in Dogs After Reticuloendothelial Blockade.*—We [2c] have previously confirmed the report of Klein and Levinson [2b] that reticuloendothelial blockade produced by the intravenous injection of india ink produces a marked retention of subsequently injected bromsulphalein. The daily injection of 20 cc. of 8 per cent india ink in saline solution for from three to eight days results in

4. Rosenthal, S. M., and Bourne, W.: Effects of Anesthetics on Hepatic Function, J. A. M. A. **90**:377 (Feb. 4) 1928.

retention of from 30 to 80 per cent of the dye during the primary stage and from 10 to 50 per cent during the secondary phase. Similar degrees of blockade result in some impairment of the removal of injected bilirubin. Not only is the retention of bilirubin less striking than that of bromsulphalein, but it occurs almost entirely in the secondary phase of the bilirubin curve.

5. *Removal of Bromsulphalein in Dogs Under the Influence of Decholin.*—Decholin (20 per cent sodium dehydrocholate) was employed as a cholagogue, as it was thought that an increased rate of excretion of bile should have a significant effect on the rate of removal of materials excreted exclusively or mainly in the blood. Negligible effects were observed when decholin was injected previous to the administration

TABLE 2.—*Summary of Data*

Condition	Bilirubin	Bromsulphalein
Normal	50 to 60% removed during primary phase; 40 to 50% during secondary phase	Rate of removal very rapid; 85 to 95% in primary phase; 5 to 15% during secondary phase
Anesthesia	Slight effect on primary phase; somewhat greater effect an secondary phase	No effect in either phase until after prolonged or deep anesthesia
Reticuloendothelial blockade	Definite impairment in secondary phase of removal; slight effect in primary phase	Marked impairment in primary phase; impairment of secondary phase
Obstruction of bile ducts (without bilirubinemia)	Immediate and complete impairment of secondary phase	No initial impairment of either phase; later impairment of secondary phase; then primary phase
Decholin	Negligible effect in primary phase; possibly some augmentation of secondary phase	Marked impairment of primary phase; slight effect on secondary phase

of bilirubin and during the ensuing two hours after the injection in the normal dog. The injection of decholin before that of bromsulphalein, however, resulted in retention of the dye. This effect was most marked during the primary phase of the curve and was usually absent in the secondary phase.

COMMENT

The available evidence indicates rather conclusively that both bromsulphalein and bilirubin when injected intravenously are ultimately almost completely excreted by the liver in the bile. Nevertheless the two substances leave the blood stream at different rates and these rates of removal from the blood stream are affected both qualitatively and quantitatively in different degrees by various procedures. The conspicuous feature of the removal of bromsulphalein from the blood stream is the abrupt fashion with which 85 to 95 per cent disappears within five minutes. It is inconceivable that this proportion of the dye is excreted so rapidly in the bile, and direct observations on the

appearance of the dye in the bile confirm this view.[3] A significant proportion (50 to 60 per cent) of the injected bilirubin likewise leaves the blood stream within five minutes, but the remaining portion (40 to 50 per cent) not only is considerably in excess of the corresponding remnant of bromsulphalein but may also be more readily associated with its excretion in the bile. Anesthesia, which depresses the secretion of bile, impairs the secondary phase of removal of bilirubin more markedly than that of bromsulphalein. Ligation of the bile ducts, which completely suppresses the secretion of bile, likewise impairs the removal of bilirubin more strikingly than it does in the case of bromsulphalein. We [5] have elsewhere presented evidence that such retention of dye as does occur after ligation of the ducts is subsequent to and related to the ensuing bilirubinemia.

Moderate to marked retention of bromsulphalein occurred in all animals in which blockade with india ink was carried out,[6] the degree generally varying directly with the postulated degree of blockage (as indicated by the number of daily injections of india ink). Although both the primary and the secondary phase of dye removal are affected, the more marked retention seems to occur during the former. This is in contradistinction to the rate of removal of bilirubin. The amount of bilirubin which is removed during the primary phase is only slightly less than that for the normal animal, and the more marked interference with removal of the pigment occurs during the secondary phase of the curve.

The intravenous administration of decholin to normal or anesthetized dogs either had no effect or appeared to facilitate the removal of bilirubin to some extent. Similar injections of decholin before injections of bromsulphalein, however, resulted in a striking retention of the injected dye. Thus, measures which suppress the secretion of bile do not correspondingly suppress the removal of bromsulphalein from the blood stream, and a measure which increases the secretion of bile not only does not enhance the removal of the dye from the blood stream but actually interferes with it. As opposed to this, measures which suppress the secretion of bile impair the removal of intravenously injected bilirubin, and, conversely, measures which increase the output of bile either facilitate the removal of the pigment from the blood stream or have no appreciable effect.

5. Dragstedt, C. A., and Mills, M. A.: Proc. Soc. Exper. Biol. & Med. **34**: 467, 1936; Am. J. Physiol. **119**:713, 1937.

6. In speaking of reticuloendothelial blockade in connection with this article, we feel that a "blockade" produced by intravenous injections of india ink may probably be considered to be no more than a temporary and incomplete reduction of function of the littoral cells of the reticuloendothelial system (those in direct contact with the blood stream) and that there is little evidence that any other cells of the system are more than slightly affected (Mills, M. A.: To be published).

CONCLUSION

It is thus clear that, used as a test of hepatic function, the rate of removal of intravenously injected bromsulphalein from the blood stream when abnormal does not indicate the same pathologic condition as would corresponding alterations in the test with bilirubin. The evidence is largely circumstantial, but it seems fairly well indicated that the removal of bromsulphalein from the blood stream is brought about chiefly by the activity of the reticuloendothelial system (of which the Kupffer cells in the liver constitute an important fraction) and that, correspondingly, disturbances in the removal of dye reflect some impairment of function in this system of cells. Aside from the initial fractional removal of bilirubin (which can probably be accounted for by diffusion into the tissue, engulfment by the reticuloendothelial system, etc.), the removal of bilirubin from the blood stream seems to be dependent on and related to its excretion in the bile, and, correspondingly, disturbances in the removal of bilirubin reflect some impairment in this function, which is presumably related to the parenchymal cells of the liver.

EXCRETION OF BILE PIGMENT AND HEPATIC FUNCTION IN DISEASES OF THE BLOOD

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The excretion of an abnormally large amount of bile pigment has long been recognized as an almost constant feature of pernicious anemia in relapse. Furthermore, the excretion of bile pigment has been found to return to normal after the institution of adequate treatment.[1] The significance of these observations has given rise to considerable controversy. One school of thought [2] holds the view that the bile pigment formed in the normal course of destruction of blood or possibly through some activity of the liver apart from that of the destruction of blood cannot be utilized for the formation of new blood in patients with pernicious anemia and hence is excreted almost quantitatively. The second school [3] regards the increased excretion of bile pigment in pernicious anemia as evidence of excessive destruction of red blood cells. In this study the metabolism of bile pigment in various forms of blood dyscrasia has been investigated, and the results in pernicious anemia have been compared with those obtained in other forms of anemia.

The quantitative determination of the urobilinogen excreted in the feces is generally accepted as the most reliable method of studying the excretion of bile pigment in human beings. Jaundice, an elevated serum

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1. (*a*) Watson, C. J.: The Average Daily Elimination of Urobilinogen in Health and in Disease, with Special Reference to Pernicious Anemia, Arch. Int. Med. **47**:698-726 (May) 1931. (*b*) Farquharson, R. F.; Borsook, H., and Goulding, A. M.: Pigment Metabolism and Destruction of Blood in Addison's (Pernicious) Anemia, ibid. **48**:1156-1185 (Dec.) 1931. (*c*) Filo, E.: Die Wirkung der Leberbehandlung auf die Bildung und Ausscheidung der Gallenfarbstoffe bei perniziöser Anämie, Folia haemat. **44**:368-384, 1931.

2. Whipple, G. H.: Pigment Metabolism and Regeneration of Hemoglobin in the Body, Arch. Int. Med. **29**:711-731 (June) 1922; Hemoglobin Construction Within the Body as Influenced by Diet Factors: Consideration of Anemia Problems, Am. J. M. Sc. **175**:721-733, 1928. Castle, W. B., and Minot, G. R.: Pathological Physiology and Clinical Description of the Anemias, New York, Oxford University Press, 1936, p. 10.

3. Krumbhaar, E. G.: Thoughts on the Morbid Processes Active in Pernicious Anemia, Am. J. M. Sc. **175**:523-527, 1928. Dock, W.: The Importance of Hemolysis in the Pathogenesis of Macrocytic Anemia, in Medical Papers Dedicated to Henry Asbury Christian, Baltimore, Waverly Press, Inc., 1936, pp. 545-558.

content of bilirubin and an increased urinary content of urobilin are all suggestive of an increase in formation and excretion of bile pigment but cannot be accepted as conclusive proof of it, since each of these three signs, alone or in any combination, may be found in the presence of hepatic disease when the total excretion of bile pigment may be normal or even subnormal. It is for that reason that in this study the average daily output of urobilinogen in the feces has been chiefly relied on as an index of the output of bile pigment. Parallel with the determinations of urobilinogen in the feces, the output of urobilinogen in the urine has been quantitated in most cases, and in order to obtain a better idea of the condition of the liver, one or more tests of hepatic function have been made in certain of the cases studied.

The two tests of hepatic function which have been employed in this study are (1) the test of bilirubin excretion and (2) the test of hippuric acid synthesis. The first, introduced by Eilbott [4] and von Bergmann [5] in 1927, has recently been developed by Harrop and Barron [6] and by Soffer and Paulson [7] in this country. Soffer [8] has stated that he regards it as the most sensitive test of the excretory function of the liver. In 1932 Quick [9] introduced a test for hepatic function based on the synthesis and excretion of hippuric acid after the oral administration of sodium benzoate. Since the synthesis depends on the conjugation of benzoic acid (an aromatic compound) with aminoacetic acid to form hippuric acid, Quick said he regards this test as a measure of the protective mechanism of the body. In view of the distinct possibility that a hemolytic toxin may play a role in the etiology of pernicious anemia, in addition to the fact that certain other types of blood dyscrasia appear to be produced by aromatic compounds (e. g., benzene, arsphenamine and aminopyrine), it was desirable to have a test of the ability of the liver to detoxify such substances.

4. Eilbott, W.: Funktionsprüfung der Leber mittels Bilirubinbelastung, Ztschr. f. klin. Med. **106**:529-560, 1927.

5. von Bergmann, G.: Zur funktionellen Pathologie der Leber insbesondere der Alkohol-Aetiologie der Cirrhose, Klin. Wchnschr. **6**:776-780, 1927.

6. Harrop, G. A., and Barron, E. S. G.: The Excretion of Intravenously Injected Bilirubin as a Test of Liver Function, J. Clin. Investigation **9**:577-587, 1931.

7. Soffer, L. J., and Paulson, M.: Comparative Advantages and Further Modification of the Bilirubin Excretion Test for Hepatic Function, Am. J. M. Sc. **192**:535-540, 1936.

8. Soffer, L. J.: Present-Day Status of Liver Function Tests, Medicine **14**:185-254, 1935.

9. Quick, A. J.: Conjugation of Benzoic Acid with Glycine, a Test of Liver Function, Proc. Soc. Exper. Biol. & Med. **29**:1204-1205, 1932; The Synthesis of Hippuric Acid: A New Test of Liver Function, Am. J. M. Sc. **185**:630-635, 1933.

METHODS

Excretion of Urobilinogen.—The content of urobilinogen in the feces and in the urine was determined quantitatively by Watson's [1a] modification [10] of Terwen's [11] original method. The method depends on reducing urobilin to urobilinogen with ferrous hydroxide and then combining the urobilinogen with paradimethylaminobenzaldehyde to produce a red solution which can be compared colorimetrically with a phenolphthalein standard.

Stools were collected over a three or four day period. Two different methods of collection were employed: (*a*) The stools were collected on waxed paper and kept in a previously weighed cardboard carton in the ice box until the end of the collection period. The carton was then weighed once more, and the total weight of the stool was thus determined. The total three or four day collection of stool was thoroughly mixed in a mortar, and a 5 Gm. aliquot was taken for determination of the urobilinogen content. (*b*) Stools were collected in large enamel buckets, which were kept in the ice box until the end of the collection period. Sufficient water was added to make up a thick suspension, which was transferred to a 2 liter glass bottle and stirred for fifteen to thirty minutes with an air-driven stirring rod. After the suspension had been rendered homogeneous, the whole was measured, and a 5 to 20 cc. aliquot was taken for the determination of urobilinogen. This second method proved more satisfactory.

Urine for the determination of urobilinogen was collected over a twenty-four to forty-eight hour period, 10 cc. of a saturated alcoholic solution of salicylic acid or 2 cc. of toluene serving as a preservative. The collection bottles were kept in the ice box.

The results are expressed in terms of the average twenty-four hour output of urobilinogen in milligrams. Watson [1a] has given the following upper limits of normal: in the feces, 250 mg. for males and 175 mg. for females; in the urine, 2 to 2.5 mg. a day.

Hepatic Function Tests.—(*a*) The bilirubin excretion test was carried out in the manner described by Harrop and Barron; in the earlier tests the colorimeter was employed, whereas more recently the potassium dichromate standards devised by Soffer and Paulson for direct comparison with the acetone-treated blood plasma have been used. The patients were given 1 mg. of bilirubin per kilogram of body weight intravenously. Retention of more than 5 per cent of the injected bilirubin after four hours is regarded as evidence of hepatic dysfunction.

(*b*) The test of hippuric acid synthesis was carried out according to the original directions of Quick, the sodium hydroxide titration being employed in preference to the more complicated ether extraction method. Although the method actually determines the amount of hippuric acid in the urine, the results are expressed in terms of benzoic acid. Quick concluded that normal persons should excrete at least 3 Gm. of benzoic acid within four hours after the ingestion of 5.9 Gm. of sodium benzoate. Thus the excretion of less than 3 Gm. of benzoic acid in four hours may be regarded as evidence of hepatic damage, provided intestinal absorption and renal function are normal.

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10. Since the present study was undertaken C. J. Watson (Am. J. Clin. Path. **6**:458, 1936) has published a further modification of the method which gives more satisfactory measurements of urobilinogen in the urine but makes little difference in determinations of this substance in the feces (Watson, C. J.: Personal communication to the author).

11. Terwen, A. J. L.: Ueber ein neues Verfahren zur quantitativen Urobilinbestimmung in Harn und Stuhl, Deutsches Arch. f. klin. Med. **149**:72-101, 1925.

Examination of the Blood.—For blood counts and hematocrit determination, 4 cc. of venous blood was placed in a small rubber-stoppered glass bottle with 0.02 cc. of a 20 per cent solution of potassium oxalate as an anticoagulant. Standardized pipets and counting chambers were employed for cell counts. The hemoglobin value was determined by the Sahli method, 100 per cent representing 14.5 Gm. per hundred cubic centimeters of blood. Reticulocytes were counted in blood smears made on cover slips previously stained with brilliant cresyl blue and subsequently counterstained with Wright's stain. Wintrobe hematocrit tubes were employed in determining the volume of packed erythrocytes, from which the mean corpuscular volume was readily calculated. The icterus index was determined by the Meulengracht method.

The fragility of the erythrocytes was determined by the usual method, 1 drop of a 50 per cent suspension of washed red blood cells being added to 2 cc. of the saline solution. Saline dilutions from 0.5 to 0.26 per cent, kept in stock bottles, were employed for routine tests; when markedly increased fragility necessitated it, fresh solutions were made up, beginning at 0.8 per cent. A control test was made with each unknown. Since the minimum resistance for controls was always at 0.46 or 0.44 per cent, hemolysis beginning at 0.48 per cent or above was regarded as signifying decreased minimum resistance (or increased fragility); hemolysis beginning at 0.42 per cent or below, as evidence of increased minimum resistance (or decreased fragility). With the control levels for maximum resistance varying from 0.36 to 0.34 per cent, complete hemolysis at 0.38 per cent or above was regarded as signifying decreased maximum resistance; complete hemolysis at 0.32 per cent or below, as evidence of increased maximum resistance.

MATERIAL

The present study included 74 patients with blood dyscrasias for whom determinations of urobilinogen were carried out. Either the bilirubin excretion test or the hippuric acid synthesis test of hepatic function or both were performed on 48 of the 74 patients. The patients were divided into five groups, according to the disorder presented, as follows: (1) pernicious anemia and sprue, (2) hemolytic anemias, (3) aplastic anemias, (4) leukemias and (5) miscellaneous conditions, which included primary hepatic disease, Banti's syndrome, polycythaemia vera and Hodgkin's disease. Complete blood counts were made for all patients, and additional data, such as erythrocyte fragility, reticulocyte counts and icterus index, were obtained in many cases. The results of these studies will be discussed separately under the individual headings.

RESULTS

Group 1: Pernicious Anemia and Sprue.—Fourteen patients make up this group: 10 with typical Addison's pernicious anemia, 1 with pernicious anemia of pregnancy, 1 with macrocytic anemia associated with intestinal anastomosis and 2 with tropical sprue.

The average daily excretion of urobilinogen in the feces was determined in 9 of the 10 cases of pernicious anemia during relapse, and in 8 of the 9 cases the values obtained were well above the upper limit of normal, the highest value being 1,050 mg. per day in case 2. One patient (case 4) in a mild relapse gave a normal value. In the 4 cases in which determinations of the urobilinogen excretion were carried out

after adequate therapy with liver extract, the values had returned to normal levels. The observation that the amount of urobilinogen excreted in the feces is as a rule elevated during a relapse of pernicious anemia and returns to normal after liver therapy confirms the findings of numerous other workers. Two patients (cases 2 and 5), who were followed during the pretreatment and post-treatment periods, well illustrate the progressive decrease in the rate of excretion of bile pigment after treatment has been instituted (charts 1 and 2). Case 2 (chart 1)

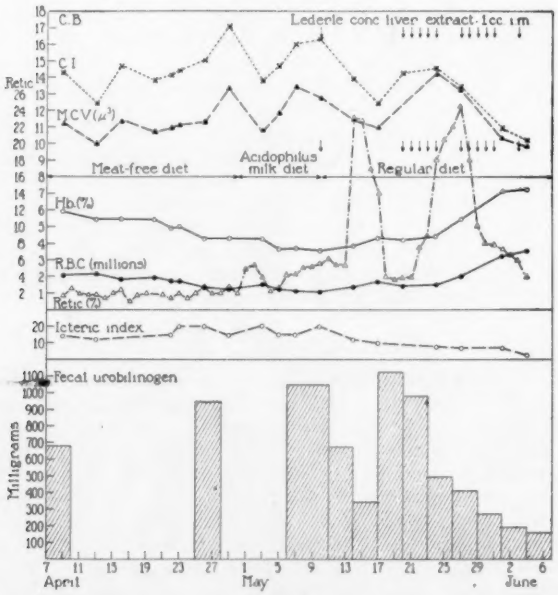

Chart 1 (case 2).—A man aged 23 with pernicious anemia. The output of urobilinogen in the feces was determined before and after therapy with liver extract. Note the transient improvement after a single injection of liver extract and the secondary rise in the reticulocyte count, with continued improvement, after intensive therapy with liver extract. In this chart the values for the color index (*C. I.*) have been multiplied by 10, and the values for the mean corpuscular volume (*M. C. V.*) in cubic microns have been divided by 10. In charts 1 to 4 the percentage values for hemoglobin (*Hb.*) have been divided by 10, and the values for erythrocytes (*R. B. C.*) have been divided by 1,000,000.

is of particular interest. The patient was given intramuscularly 1 cc. of a concentrated liver extract, the amount derived from 100 Gm. of whole liver. The reticulocyte count rose to 23 per cent on the fifth day after the injection, and there was a slight improvement in the blood

values coincident with a distinct decrease in the level of urobilinogen in the feces. This was only transient, for ten days later the excretion had again risen to the pretreatment level. With more intensive parenteral therapy with liver extract a second rise in the reticulocyte count occurred, and the urobilinogen content of the feces gradually dropped to a normal level, as did the icterus index; coincident with these changes the erythrocyte count and the hemoglobin value rose rapidly, while the color index and the mean corpuscular volume fell toward normal. It is of some interest that the output of urobilinogen remained well above

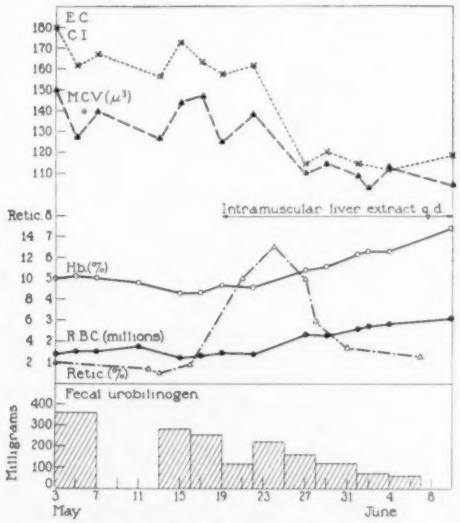

Chart 2 (case 5).—A woman aged 55 with pernicious anemia. Note the return of the output of urobilinogen to normal after intensive therapy with liver extract. In this chart the values for the color index (*C. I.*) have been multiplied by 100.

normal throughout the period of the reticulocyte response, that is, at a time when cell production and new hemoglobin formation were going on at a maximum rate. This observation, which is scarcely compatible with the theory of nonutilization, suggests that hemolysis was going on at an abnormally rapid rate even after active erythropoiesis had set in. In case 6 a similar situation was presented, with the output of pigment remaining well above normal even after a maximum reticulocyte response of 60 per cent.

Chart 2 also illustrates the progressive drop in the output of urobilinogen after the institution of therapy with liver extract in a case of relatively mild pernicious anemia.

Summary of Data

Case; Initials; Sex; Age	Diagnosis	Date	Erythrocytes, Millions	Hemoglobin, %	Leukocytes, Thousands	Color Index	Mean Corpuscular Volume, Cu. Microns	Reticulocytes, %	Fragility, %	Icteric Index	Serum Bilirubin, Mg. per 100 Cc.	Hepatic Function Tests: 4 Hour Bilirubin Retention, %	Hepatic Function Tests: 4 Hour Benzoic Acid Excretion, Gm.	Urobilinogen Excretion, Mg. per Day: Fecal	Urobilinogen Excretion, Mg. per Day: Urinary	Comment
							Group I. Pernicious Anemia and Sprue									
1. J. B. M, 40	Pernicious anemia	3/ 3/36	2.34	62	6.4	1.32	107	1.2	.....	15	1.16	0	....	275	2.52	Before liver
2. C. B. M, 23	Pernicious anemia	4/10/37	2.06	59	2.8	1.43	112	1.8	0.46-0.36	14	2.00	0	3.51	680	23.00	Before liver
		5/11/37	1.10	36	2.9	1.64	127	6.2	.....	15	....	..	....	1,050	20.90	Before liver
		6/ 7/37	3.54	72	5.2	1.02	98	4.0	.....	3	....	..	....	163	....	After liver (chart 1)
3. C. B.* M, 64	Pernicious anemia	7/14/36	1.27	36	2.1	1.42	122	1.5	0.46-0.34	12	1.89	60	....	369	1.26	Before stomach U.S.P.
		7/30/36	....	..	...	....	...	...	.....	..	1.02	5	3.73	...	....	After stomach U.S.P.
4. M. C. F, 36	Pernicious anemia	2/ 2/37	2.80	89	6.7	1.60	127	0.8	.....	5	0.30	10	1.08	149	0.32	Before liver
5. E. C. F, 55	Pernicious anemia	5/ 7/37	1.50	50	4.4	1.67	140	1.4	.....	5	....	..	....	360	1.10	Before liver
		6/ 4/37	2.82	63	5.9	1.12	114	2.4	.....	6	....	..	....	76	....	After liver (chart 2)
6. G. C. M, 61	Pernicious anemia	2/ 2/37	0.48	20	3.4	2.09	186	1.1	.....	8	1.50	0	1.47	442+	11.80	Before liver
		2/17/37	....	..	...	....	...	60.0	.....	..	....	..	....	...	....	Began liver, intramuscularly on 2/12
		2/20/47	1.92	42	3.9	1.09	118	20.0	.....	..	....	..	....	533	0.54	After liver
		2/26/37	2.17	57	2.8	1.30	126	5.0	.....	3	....	..	....	28	Trace	After liver
		3/ 4/37	2.90	67	5.1	1.15	107	2.0	.....	..	....	..	....	52	Trace	After liver
		3/10/37	3.76	78	5.6	1.04	97	1.4	.....	5	....	..	2.05	93	0.37	After liver
7. F. G. M, 54	Pernicious anemia	11/14/35	2.12	71	3.6	1.68	130	1.4	.....	..	....	..	....	305	....	Before liver therapy
8. M. L. F, 65	Pernicious anemia	5/12/36	1.13	26	1.6	1.15	129	4.0	.....	15	2.01	..	....	...	2.75	Before liver therapy; patient too constipated for satisfactory stool collection
9. C. M. F, 71	Pernicious anemia	3/23/37	1.22	32	2.2	1.31	122	2.0	.....	15	0.60	0	....	300	0.58	Before liver therapy
		5/15/37	3.20	58	6.1	0.91	92	1.0	.....	6	....	..	....	70	....	Two weeks after liver therapy
		5/20/37	3.38	63	7.4	0.93	92	1.4	.....	4	....	..	....	38	....	Three weeks after liver therapy
10. E. T. M, 48	Pernicious anemia	10/22/36	1.23	40	3.6	1.63	147	...	.....	..	3.00	44	....	...	....	Before liver therapy
		11/27/36	2.49	64	3.7	1.28	128	...	.....	10	1.63	0	....	201	12.72	Partial remission after liver extract; patient constipated
		12/ 2/36	....	..	...	....	...	...	.....	..	....	..	3.15	295	1.95	Bowels regulated
11. A. C.	Pernicious	2/26/37	2.86	74	8.9	1.30	114	1.2	.....	3				46	Trace	Before liver therapy

11. A. C. F, 31	Pernicious anemia of pregnancy	2/26/37	2.86	74	8.9	1.30	114	1.2	.....	3	....	..	....	66	Trace	Before liver therapy
12. D. S. M, 56	Macrocytic anemia; intestinal anastomosis	1/ 9/36	2.13	60	2.4	1.41	131	2.0	.....	4	0.50	0	1.86	...	0.24	Before treatment; diarrhea present
		2/19/37	4.88	88	7.1	0.90	82	1.0	.....	5	0.30	0	3.45	...	....	After treatment with liver extract and diet for sprue for 10 mo.
13. M. H. M, 45	Tropical sprue	10/21/36	4.34	90	3.5	1.03	91	0.6	.....	5	0.50	5	2.75	3	Trace	Stools pasty and gray
		10/28/36	....	..	...	....	...	...	.....	..	....	..	....	61	Trace	Stools pasty but better color
		11/11/36	....	..	...	....	...	...	.....	..	....	..	....	30	0.87	Stools pasty but better color
		12/ 5/36	....	..	...	....	...	...	.....	..	....	..	....	23	0.49	Stools pasty but better color
14. D. K. F, 47	Tropical sprue	11/ 4/36	4.47	88	6.6	1.00	89	...	.....	5	....	..	....	107	Trace	Balanced diet; no liver extract
		12/ 5/36	....	..	...	....	...	...	.....	..	....	..	....	54	Trace	Low protein diet; soft gray-green stools
		12/19/36	....	..	...	....	...	...	.....	..	....	..	....	83	....	After liver extract and diet for sprue; normal stools
							Group 2.	Hemolytic	Anemias							
15. J. C. F, 7	Congenital hemolytic jaundice	1/24/36	1.50	36	4.6	1.20	97	15.8	0.70-0.40	20	....	..	....	500	0.36	Before splenectomy
		5/19/36	5.04	85	6.5	0.82	85	0.2	0.60-0.38	4	....	..	....	36	0.63	Two weeks after splenectomy
		5/15/37	4.96	81	13.1	0.82	70	5.5	0.55-0.40	3	....	..	....	67	....	One year after splenectomy; acute rheumatic fever
16. M. S. F, 17	Congenital hemolytic jaundice	11/ 6/36	4.28	98	9.8	1.13	86	11.3	0.70-0.40	30	....	..	3.18	622+	5.78	Before splenectomy
		11/11/36	4.26	92	8.0	1.08	84	10.9	.....	35	....	..	....	1,087	3.84	Before splenectomy
		12/11/36	4.21	95	8.1	1.13	93	1.5	0.55-0.38	6	....	..	....	127	Trace	Two weeks after splenectomy
		6/ 8/37	4.72	110	10.7	1.16	96	1.1	0.54-0.38	12	....	..	....	127	0.30	Six months after splenectomy
17. E. W. M, 5	Congenital hemolytic jaundice	6/17/37	2.66	60	7.0	1.13	83	24.0	0.52-0.38	25	....	..	....	793	1.11	Before splenectomy
		7/ 9/37	5.04	88	13.7	0.85	82	0.8	.....	6	....	..	....	18	0.15	Two weeks after splenectomy
18. P. N. M, 6	Hemolytic anemia, Cooley's type (?)	4/ 6/37	3.67	55	5.2	0.75	65	9.0	0.42-0.26	50	....	..	....	520	1.09	Before splenectomy
		4/ 9/37	3.44	52	5.1	0.76	71	9.0	.....	25	....	..	....	350	....	Before splenectomy
		5/ 9/37	3.65	70	7.6	0.96	82	2.0	0.42-0.26	8	....	..	....	59	....	Two weeks after splenectomy
		5/15/37	3.68	66	12.1	0.90	80	6.5	.....	8	....	..	....	63	Trace	Three weeks after splenectomy
19. A. D. M, 67	Acquired hemolytic anemia	2/ 5/37	1.81	53	6.5	1.46	132	9.8	0.50-0.38	15	1.63	0	3.90	1,517	5.64	No response to liver therapy
20. M. L. M, 23	Hemolytic anemia and purpura	7/22/36	2.18	51	2.4	1.17	101	12.0	0.44-0.34	9	....	..	....	570	1.54	Benzene poisoning; hyperplastic marrow; later complete recovery

* Father of the patient in case 2.

Summary of Data—Continued

Case; Initials; Sex; Age	Diagnosis	Date	Erythrocytes, Millions	Hemoglobin, %	Leukocytes, Thousands	Color Index	Mean Corpuscular Volume, Cu. Microns	Reticulocytes, %	Fragility, %	Icteric Index	Serum Bilirubin, Mg. per 100 Cc.	Hepatic Function Tests: 4 Hour Bilirubin Retention, %	Hepatic Function Tests: 4 Hour Benzoic Acid Excretion, Gm.	Urobilinogen Excretion, Mg. per Day: Fecal	Urobilinogen Excretion, Mg. per Day: Urinary	Comment
							Group 2. Hemolytic Anemias—Continued									
21. C. D. M, 38	Hemolytic anemia and purpura	1/11/36	2.78	63	6.9	1.13	98	3.0	0.46-0.34	10	....	..	3.15	556	1.54	Marrow picture identical with that in case 20
		2/ 7/36	2.00	46	33.6	1.15	99	12.8	.....	15	....	..	....	1,000	....	Patient later died at another hospital with typical picture of acute myelogenous leukemia
							Group 3. Aplastic Anemias									
22. M. A. F, 36	Aplastic anemia	2/22/36	1.70	46	2.1	1.35	...	...	.....	4	....	..	....	129	0.92	Hypoplastic fatty marrow
23. V. B. F, 18	Aplastic anemia	7/18/36	1.58	33	1.6	1.04	88	0.5	.....	6	....	..	....	149	1.82	Hypoplastic fatty marrow
24. S. B. M, 44	Aplastic anemia due to benzene	11/ 1/35	0.60	12	1.1	1.00	80	1.0	.....	4	....	..	....	85	....	Hypoplastic fatty marrow
25. R. B. F, 27	Aplastic anemia due to arsphenamine	10/30/36	1.21	30	1.9	1.24	100	1.6	.....	10	....	6	2.64	141	16.60	Hypoplastic fatty marrow
26. J. B. F, 59	Aplastic anemia due to hair dye (?)	12/30/35	1.21	31	2.1	1.28	105	4.0	0.46-0.36	6	....	..	3.60	230	2.72	Hypoplastic fatty marrow; presplenectomy values
		3/25/36	1.99	46	2.2	1.15	103	2.8	.....	5	....	..	....	60	1.92	Six weeks after splenectomy
		10/13/36	0.82	20	4.6	1.22	123	1.7	.....	5	0.40	9	....	20	1.06	Eight months after splenectomy
27. A. C. M, 19	Aplastic anemia due to hydroquinone (?)	3/20/27	0.65	17	2.8	1.30	128	4.8	0.44-0.34	6	....	..	....	108	0.94	Hypoplastic fatty marrow; 3 day period before transfusion
		3/23/37	0.67	19	1.6	....	...	2.4	.....	..	....	..	....	600	....	Three day period after transfusion (chart 3)
28. H. D. F, 66	Aplastic anemia	12/10/35	1.83	40	2.8	1.09	94	0.2	.....	3	....	..	3.62	86	....	Hypoplastic fatty marrow
29. F. H. M, 59	Aplastic anemia due to benzene (?)	5/ 8/36	1.06	27	3.1	1.27	119	3.5	0.44-0.34	3	....	..	....	83	0.97	Hypoplastic fatty marrow

30. M. J. F, 43	Aplastic anemia	6/15/37	1.30	35	1.3	1.35	106	2.3	.....	7	....	..	....	189	Trace	
31. T. M. M, 54	Aplastic anemia	11/ 1/35	0.86	19	1.9	1.10	110	3.6	0.48-0.38	15	....	..	....	463	....	Hypoplastic fatty marrow
32. E. S. F, 49	Aplastic anemia due to benzene	3/16/37	2.52	63	5.9	1.25	104	3.7	0.46-0.36	7	0.40	0	3.77	232	Trace	Hypoplastic fatty marrow
		4/30/37	1.79	51	3.7	1.42	109	4.0	.....	5	....	..	....	197	....	
33. E. T. M, 68	Aplastic anemia; miliary tuberculosis	5/19/36	2.47	51	1.5	1.03	96	...	.....	5	0.38	5	1.92	62	1.85	Normally cellular marrow with arrest of maturation
34. S. B. F, 25	Aplastic anemia	10/30/36	2.64	57	3.9	1.08	96	2.2	.....	5	0.39	8	....	24	0.54	Immature cellular marrow
		1/28/37	3.30	63	10.5	0.95	98	...	.....	2	0.25	9	3.66	...	....	
35. A. D. M, 53	Aplastic anemia	11/14/35	1.19	21	1.3	0.88	84	1.6	.....	..	....	..	....	272	....	Immature cellular marrow
36. W. G. M, 26	Aplastic anemia	11/11/36	2.00	40	2.4	1.00	...	0.8	0.42-0.28	..	0.25	6	....	272	1.25	Immature cellular marrow; hemochromatosis
37. D. L. M, 26	Aplastic anemia	4/28/37	1.40	35	1.1	1.25	97	4.2	0.42-0.32	5	0.35	3	3.90	212	0.55	First urobilinogen determination, 5 days after transfusion, with chill
		5/ 7/37	1.23	29	1.5	1.18	94	2.1	.....	5	....	..	....	76	....	Second determination, 2 weeks after transfusion; immature cellular marrow with large hyperplastic nodules
38. G. M. M, 34	Aplastic anemia due to roentgen rays (?)	9/30/36	1.31	26	1.0	1.00	90	3.8	0.44-0.34	..	1.57	0	3.31	500	1.05	Immature cellular marrow
		12/ 2/36	1.43	31	0.9	1.09	93	2.5	.....	6	....	..	....	380	3.32	
		3/ 1/37	1.67	30	1.1	0.90	79	2.3	0.44-0.34	4	....	..	....	450	1.44	Before splenectomy
		6/15/37	1.17	26	3.3	1.11	...	2.5	.....	..	....	..	....	177	1.69	Seven weeks after splenectomy
		6/22/37	0.88	19	4.4	1.08	119	...	.....	5	....	..	....	230	16.08	Eight weeks after splenectomy; 5 weeks after transfusion (chart 4)
39. F. M. M, 47	Aplastic anemia	5/ 7/37	3.23	84	2.5	1.30	110	1.4	.....	5	0.40	0	3.50	120	0.81	Immature cellular marrow
40. R. M. M, 21	Aplastic anemia; hemochromatosis	11/ 1/35	1.29	21	2.5	0.82	102	...	.....	..	0.85	9	2.20	172	....	Immature cellular marrow; hemochromatosis
41. C. S. F, 53	Aplastic anemia due to hair dye (?)	3/16/37	1.60	40	2.3	1.25	92	1.0	0.44-0.34	5	0.40	0	3.60	133	Trace	Immature cellular marrow
		4/ 2/37	1.84	37	1.4	1.00	91	0.4	.....	8	....	..	....	104	....	

Summary of Data—Continued

Case; Initials; Sex; Age	Diagnosis	Date	Erythrocytes, Millions	Hemoglobin, %	Leukocytes, Thousands	Color Index	Mean Corpuscular Volume, Cu. Microns	Reticulocytes, %	Fragility, %	Icteric Index	Serum Bilirubin, Mg. per 100 Cc.	Hepatic Function Tests: 4 Hour Bilirubin Retention, %	Hepatic Function Tests: 4 Hour Benzoic Acid Excretion, Gm.	Urobilinogen Excretion, Mg. per Day: Fecal	Urobilinogen Excretion, Mg. per Day: Urinary	Comment
Group 3. Aplastic Anemias—Continued																
42. P. S. M, 60	Aplastic anemia	5/24/37	1.76	47	4.3	1.33	102	1.8	.....	2	....	..	....	265	....	Immature cellular marrow
43. F. Y. F, 30	Aplastic anemia	12/ 9/36	2.21	66	2.8	1.49	130	2.3	0.44-0.34	5	0.75	0	3.08	98	1.02	Immature cellular marrow
44. J. L. M, 47	Aplastic anemia	1/26/37	1.03	24	1.5	1.17	104	3.6	0.44-0.34	3	0.60	4	3.25	235	9.50	Hodgkin's type of marrow
		2/12/37	....	..	...	....	...	...	.....	..	....	..	....	...	2.00	
45. A. P. M, 58	Aplastic anemia	3/24/37	3.12	69	1.3	1.10	86	4.0	.....	100	2.00	0	2.21	52	23.00	Hodgkin's disease of liver, spleen and bone marrow
46. A. L. M, 18	Aplastic anemia	2/19/36	1.64	36	5.7	1.10	93	1.0	0.46-0.34	6	0.52	21	3.61	178	7.10	Sclerotic bone marrow; marked hepatosplenomegaly
		10/13/36	1.52	26	3.6	0.86	84	...	.....	6	0.29	6	....	175	16.85	
		11/ 6/36	1.69	32	1.0	0.95	97	...	.....	4	....	..	....	...	27.70	
Group 4. Leukemias																
47. C. D. M, 65	Chronic myelogenous leukemia	4/30/37	2.68	50	172.0	0.93	87	7.6	.....	8	0.35	0	2.44	298	0.95	
48. J. H. F, 18	Chronic myelogenous leukemia	12/16/36	3.42	70	212.0	1.02	82	3.5	.....	5	....	..	....	23	0.89	
49. F. F. F, 56	Aleukemic myelogenous leukemia	6/23/36	1.03	28	6.4	1.36	121	3.2	.....	14	....	..	....	169	8.70	
50. E. G. M, 63	Aleukemic myelogenous leukemia	4/15/37	1.22	35	0.6	1.43	118	3.4	.....	3	0.50	23	2.83	75	0.17	
51. V. G. F, 48	Aleukemic myelogenous leukemia due to hair dye (?)	7/10/36	1.00	32	3.0	1.60	125	1.2	.....	3	0.52	0	....	32	0.43	
52. E. H. F, 72	Aleukemic myelogenous leukemia	5/26/36	1.60	33	2.7	1.03	80	0.2	.....	3	0.38	4	....	101	Trace	

53. D. M. M, 44	Aleukemic myelogenous leukemia	3/ 3/36	1.39	42	11.8	1.51	124	6.2	0.48-0.38	8	....	..	....	560	Trace	
54. R. T. M, 15	Aleukemic myelogenous leukemia	5/15/36	4.00	82	2.4	1.03	88	...	.....	..	0.67	13	2.63	104	0.92	
55. I. B. M, 31	Chronic lymphatic leukemia	6/ 8/37	2.74	60	16.9	1.10	88	1.6	.....	5	0.55	9	3.66	210	2.30	
						Group 5. Miscellaneous Conditions										
56. H. P. F, 70	Nutritional hypochromic microcytic anemia	6/26/36	3.80	59	4.8	0.78	77	4.0	.....	2	....	..	....	67	0.35	
57. J. S. M, 59	Carcinoma of stomach; hypochromic microcytic anemia	6/10/37	3.00	39	7.3	0.65	...	...	.....	..	....	..	....	87	0	
58. V. L. F, 16	Banti's syndrome	10/14/36	4.14	70	2.4	0.85	75	2.5	0.44-0.30	3	0.45	0	1.99 2.25	103	0.16	Before splenectomy; platelets, 58,000
		12/16/36	5.10	85	8.0	0.83	...	...	.....	..	0.20	0	3.06	42	0.17	Four weeks after splenectomy; platelets, 1,100,000
59. B. L. M, 64	Banti's syndrome	12/19/36	4.60	80	2.8	0.87	...	1.8	.....	3	0.40	0	3.05	256	2.60	Platelets, 106,000
60. L. S. F, 23	Banti's syndrome	3/ 3/36	3.17	45	2.0	0.71	65	2.8	0.46-0.28	6	0.98	33	2.52	56	0.86	Before iron
		3/31/36	3.98	78	2.0	0.98	92	2.0	.....	10	....	17	....	24	0.37	Three weeks after starting iron
		10/21/36	2.14	50	2.9	1.17	89	1.4	.....	10	1.37	34	2.68	51	0.37	One week after second large hematemesis
61. N. S. F, 33	Banti's syndrome; hemangioma of spleen	2/22/36	4.18	67	4.1	0.80	75	1.6	.....	4	0.31	0	2.53	98	....	Before splenectomy; platelets, 60,000
62. E. T. M, 20	Banti's syndrome	9/16/36	3.73	59	5.9	0.79	78	2.4	0.44-0.30	5	1.10	0	2.92	132	3.07	Before iron
		10/28/36	5.24	84	3.1	0.80	82	...	.....	8	....	..	3.28	...	2.83	After iron
		1/18/37	4.83	73	7.5	0.76	...	1.0	.....	..	0.40	14	3.59	76	0.87	Two months after splenectomy and recovery from severe postoperative hemorrhage
63. M. C. F, 14	Thrombopenic purpura	2/12/37	4.76	81	8.2	0.85	90	2.4	.....	4	0.25	0	3.55	98	0.34	Platelets, 44,000
64. R. G. M, 59	Polycythaemia vera	6/16/36	4.56	94	10.5	1.03	91	3.0	.....	8	....	..	....	298	Trace	

Summary of Data—Continued

Case; Initials; Sex; Age	Diagnosis	Date	Erythrocytes, Millions	Hemoglobin, %	Leukocytes, Thousands	Color Index	Mean Corpuscular Volume, Cu. Microns	Reticulocytes, %	Fragility, %	Icteric Index	Serum Bilirubin, Mg. per 100 Cc.	Hepatic Function Tests: 4 Hour Bilirubin Retention, %	Hepatic Function Tests: 4 Hour Benzoic Acid Excretion, Gm.	Urobilinogen Excretion, Mg. per Day: Fecal	Urobilinogen Excretion, Mg. per Day: Urinary	Comment
							Group 5. Miscellaneous—Continued									
65. A. P. F, 48	Polycythaemia vera	5/26/36	8.12	168	7.9	1.03	93	1.6	0.46-0.32	6	....	..	....	156	Trace	Before treatment
		6/ 6/36	6.85	146	9.2	1.07	102	...	.....	..	....	..	....	121	Trace	After venesection
		6/19/36	7.40	160	9.9	1.08	97	...	.....	..	....	..	....	167	0.55	After acetylphenylhydrazine, 0.1 Gm. four times daily for 1 week
		7/18/36	6.70	142	10.8	1.06	95	...	.....	..	....	..	....	296	Trace	After phenylhydrazine hydrochloride, 0.1 Gm. four times daily for 11 days
66. O. B. F, 44	Cirrhosis of liver	3/14/36	2.30	58	6.6	1.26	111	...	.....	20	2.32	45	....	80	8.72	
		5/27/36	3.05	70	4.6	1.15	100	...	.....	15	....	16	....	89	5.60	
		9/30/36	2.96	72	6.0	1.22	108	...	.....	..	1.39	2	1.48	76	5.60	
		11/ 6/36	2.95	72	4.9	1.22	108	...	.....	15	1.25	20	1.73	...	10.70	
		2/12/37	3.08	66	4.6	1.07	106	...	.....	15	....	..	....	...	9.24	
67. F. K. F, 33	Cirrhosis of liver; cholangitis	3/10/37	3.53	82	5.0	1.16	98	3.0	0.42-0.28	50	....	..	1.75	153	24.60	
68. S. M. F, 50	Cirrhosis of liver	5/11/37	3.21	62	5.7	0.97	85	2.4	.....	5	0.25 0.20	9 6	1.86 2.04	58	0.39	
69. R. P. M, 13	Cirrhosis of liver; nephritis	2/ 1/36	2.31	43	9.9	0.93	82	...	.....	15	1.60	33	0.84	34	4.53	
70. P. R. M, 41	Chronic arsenic poisoning; cirrhosis of liver (?)	2/ 5/37	4.17	88	6.2	1.05	88	4.9	0.42-0.30	7	0.50	5	3.66	89	1.65	
71. M. W. M, 62	Cirrhosis of liver	1/ 9/36	2.22	50	5.9	1.12	101	0.6	0.46-0.32	4	....	..	2.67 1.74	...	0.81	
72. M. M. M, 35	Abdominal Hodgkin's disease	6/15/37	4.20	89	2.5	1.06	88	1.2	.....	5	....	..	....	176	1.34	
73. N. S. M, 26	Gaucher's disease	5/12/36	4.84	90	2.9	0.93	90	...	.....	..	1.35	0	....	220	1.28	
74. W. A. F, 25	Chronic nephritis; uremia	11/ 4/36	3.30	75	10.6	1.14	92	4.0	.....	..	....	..	...	55	....	Nonprotein nitrogen, 62 mg. per 100 cc.; urea clearance, 11 per cent of normal

The excretion of urobilinogen in the urine was increased in 5 cases and was normal in 4 cases of pernicious anemia in relapse. In case 6, when the urobilinogen in the urine was followed before and after therapy with liver extract, the level fell rapidly to a low normal value.

The patient with pernicious anemia of pregnancy (case 11) showed low values for urobilinogen in both feces and urine.

In the case of macrocytic anemia associated with intestinal anastomosis (case 12) it was impossible to obtain satisfactory collections of stool because of diarrhea. However, the low icterus index and the low value for urobilinogen in the urine may be regarded as evidence against excessive hemolysis.

Two patients with tropical sprue gave low values for urobilinogen in the feces at a time when the stools were pasty and gray-white but not actively diarrheal. For 1 patient (case 13) an average daily value of only 3 mg. of urobilinogen was obtained for the feces, with only a trace of urobilinogen appearing in the urine. A qualitative test for unchanged bilirubin gave a negative reaction. Since there was no jaundice or other evidence of retention of bile, it seemed probable that the pigment was being excreted in a nondetectable form. Manson-Bahr [12] has stated that sprue stools have a light color, not because of a decrease in the amount of bile pigment excreted but because the pigment is excreted in a colorless reduced state as urobilinogen. The correctness of this hypothesis is open to question: (1) because sprue stools tend to maintain their light color even after exposure to air and sunlight, whereas urobilinogen should be oxidized to red-brown urobilin under such conditions; (2) because such an exceedingly low value for bile pigment in typical sprue stools has been obtained with a method designed for determination of the urobilinogen quantitatively.

Bilirubin excretion tests of hepatic function were performed on 7 of the 10 patients with pernicious anemia. Three patients showed a four-hour retention of more than 5 per cent; however, since the original bilirubin level of the serum was above 1 mg. per hundred cubic centimeters in 2 of these cases, extreme caution must be taken in interpreting the results as evidence of hepatic damage.[8] It is somewhat puzzling that there was no retention of bilirubin in 4 cases in which the original bilirubin level of the serum was above 1 mg. per hundred cubic centimeters. The patient with the intestinal anastomosis and 1 patient with tropical sprue excreted bilirubin normally.

Variable results were obtained with the hippuric acid synthesis test for this group of patients. Three patients with pernicious anemia (2 had not been treated and 1 had been) gave normal results, whereas

12. Manson-Bahr, P. H.: Manson's Tropical Diseases, ed. 10, Baltimore, William Wood & Company, 1936.

2 gave low results. For 1 of the latter (case 6) the amount of hippuric acid excreted rose somewhat after the administration of liver extract but still remained well below normal. Fouts, Helmer and Zerfas [13] found a reduction in the excretion of hippuric acid in the majority of their cases of pernicious anemia in relapse, with variable improvement after treatment. The excretion of hippuric acid was somewhat lower than normal in 1 case of tropical sprue (case 13) and was even lower in the case of macrocytic anemia associated with intestinal anastomosis. Poor absorption of the sodium benzoate from the intestinal tract may well have been a factor in these 2 cases. In the latter case a normal result was obtained after the diarrhea had been controlled by appropriate therapy.

Group 2: Hemolytic Anemias.—The excretion of urobilinogen was studied in 7 cases of hemolytic anemia—3 cases of typical congenital hemolytic jaundice, 1 case of questionable erythroblastic anemia (case 18), 1 case of severe chronic hemolytic anemia of undetermined etiology (case 19) and 2 cases of acute hemolytic anemia presumably due to benzene poisoning. Well marked anemia was present in 6 of the 7 cases. All 7 cases were characterized by jaundice, splenomegaly, a high icterus index and reticulocytosis; increased fragility of the red blood cells was present in the 3 cases of congenital hemolytic jaundice and in the case in which chronic hemolytic anemia developed late in life.

The striking feature in all 7 cases was a marked increase in urobilinogen in the feces, varying from 350 mg. a day in case 18 (a boy of 6 years) to 1,517 mg. a day in case 19. The urobilinogen in the urine was definitely increased in only 2 cases.

Splenectomy was performed in the 3 cases of congenital hemolytic jaundice; in each case the urobilinogen in the feces dropped to a low normal value within two weeks after operation. The output of urobilinogen was still within normal limits one year later in case 15 and six months later in case 16. Coincident with the drop in urobilinogen value, the erythrocyte count and the hemoglobin level rose, and the icterus index and the reticulocyte count fell to normal. Erythrocyte fragility remained abnormal, although there was a tendency for the minimum resistance to return toward normal in cases 15 and 16.

Case 18 was that of a Greek boy aged 6, who presented a clinical picture of jaundice, hypochromic microcytic anemia, hyperbilirubinemia, reticulocytosis and increased output of pigment with decreased fragility of the erythrocytes, in contrast to the cases of congenital hemolytic icterus. The absence of nucleated red cells in the peripheral blood and the lack of characteristic skeletal changes cast some doubt on the diag-

13. Fouts, P. J.; Helmer, O. M., and Zerfas, L. G.: The Secretion of Hippuric Acid in Pernicious Anemia, Am. J. M. Sc. **193**:647-652, 1937.

nosis of erythroblastic anemia, although the sternal bone marrow presented a picture of normoerythroblastic hyperplasia. After treatment with liver and iron preparations had failed to improve the blood picture during a one year period of observation, it was decided to perform splenectomy in the hope of decreasing the rate of hemolysis. The immediate result was gratifying, as shown in the accompanying table; however, the erythrocyte count, hemoglobin value and reticulocyte percentage gradually returned to preoperative levels, and the icterus index slowly rose after the patient was discharged from the hospital. The urobilinogen determination three weeks postoperatively was essentially normal, suggesting that splenectomy had stopped temporarily the excessive hemolysis. However, the subsequent course made it appear that other organs had taken over the function of red blood cell destruction; unfortunately it was not possible to carry out further studies of the excretion of pigment to confirm this impression.

The bilirubin excretion test of hepatic function was performed on only 1 patient of the group (case 19), in whom hepatic damage was suspected. Although the serum bilirubin value was 1.63 mg. per hundred cubic centimeters, there was no retention of the injected bilirubin after four hours. This result is comparable to that obtained in certain cases of pernicious anemia in relapse.

The hippuric acid test gave normal results for the 3 patients tested (cases 16, 19 and 21).

Group 3: Aplastic Anemias.—Aplastic anemia is used here in the broad sense to include anemia that is refractory to any known form of therapy in which the bone marrow is (1) hypoplastic to aplastic, (2) cellular but immature, (3) of Hodgkin's type or (4) sclerotic. Twenty-five patients make up this group.

One or more determinations of the output of urobilinogen in the feces were made in all 25 cases, and the output of urobilinogen in the urine was determined in 19 of the cases. In only 8 of the 25 cases were the values for urobilinogen in the feces above Watson's upper limit of normal, and in 6 of the 8 the increase was slight. In the great majority of the cases the values were well within normal limits; abnormally low values were rarely encountered. Urobilinogen in the urine was within normal limits in 13 of the 19 cases studied; in 2 cases (cases 26 and 38) there was elevation of the urobilinogen in the urine proportional to the increase in urobilinogen in the feces, whereas in 4 cases (cases 25, 44, 45 and 46) there was a well marked increase in urobilinogen in the urine, with normal values for the feces. The increased urobilinuria suggested hepatic damage in these 4 cases, and this suggestion was borne out by further evidence of hepatic dysfunction in 3 of the 4.

The output of urobilinogen in the feces was followed over a prolonged period in 1 case (case 27) of aplastic anemia in which there was hypoplastic fatty marrow (chart 3). A sharp increase in the rate of excretion of urobilinogen followed each blood transfusion. It had been noted that the improvement in the blood picture following transfusion was transient, an observation which suggested that the patient was hemolyzing transfused blood. The urobilinogen values before and after a transfusion supported this hypothesis. On one occasion the average daily value for urobilinogen in the feces rose from 108 mg. during the three-day period before the transfusion to 600 mg. during the corresponding period immediately after the transfusion. The excretion of pigment then decreased gradually to the original level until after the

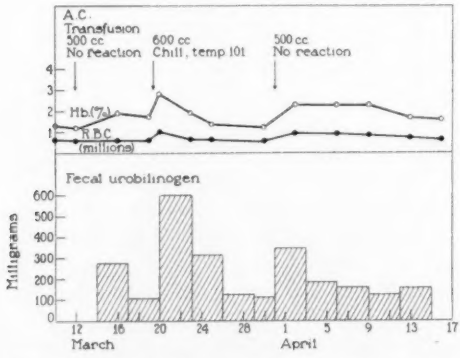

Chart 3 (case 27).—A man aged 19 with aplastic anemia showed hypoplastic bone marrow. The chart illustrates the striking increase in the output of urobilinogen following transfusions.

next transfusion. This was followed by a sharp though less pronounced rise in the urobilinogen in the feces to 350 mg. a day. The chart shows that the improvement in the hemoglobin level was maintained over a longer period after the second transfusion, apparently because less of the transfused blood was lost through hemolysis. Since there was a febrile reaction to the first transfusion and no reaction to the second, it is conceivable that either fever or the sudden hemolysis of a large number of the transfused cells may have been responsible for the increased excretion of pigment. At each transfusion whole blood was given by the direct multiple syringe method, 2 different donors of the same blood group as the patient being used. In view of the increase in output of pigment following a blood transfusion, it is obviously advisable to wait at least a week after a transfusion before beginning to collect stool for study of the urobilinogen; otherwise a false high value

may be obtained. As a further illustration of this principle, in case 37 two determinations of urobilinogen in the feces were carried out. The first collection period ran from the second to the fifth day after a transfusion, and a rather high average daily value, 212 mg., was obtained. During a second collection period, two weeks after the transfusion, the average output was much lower, 76 mg. a day.

The details of the clinical course of this group of patients will be discussed elsewhere.[14] However, the 2 patients of the group who were subjected to splenectomy (cases 26 and 38) may be briefly mentioned here. In each case the presence of abnormally large amounts of urobilinogen in the feces suggested that a hemolytic factor was playing a role in the production of the anemia. After various forms of medical therapy had proved ineffective, it seemed justifiable to remove the spleen in the hope of decreasing the rate of hemolysis. In case 26 the output of urobilinogen dropped from a preoperative level of 230 mg. a day to 60 mg. six weeks postoperatively. The patient was able to go five months without a transfusion, whereas for six months prior to operation she had required one transfusion a month. Eight months after splenectomy the value for urobilinogen in the feces was extremely low, 20 mg. a day. Thus splenectomy appeared to remove a hemolytic factor and thereby to bring about temporary improvement, yet the ultimate outcome of the disease was the usual one, with death occurring from intercurrent infection nine months after the operation.

The other splenectomized patient (case 38) has not been observed over a sufficiently long postoperative period to allow of definite conclusions as to the value of the operation. He was first followed over a period of six months, during which therapy with various hemopoietic agents failed to modify the course of his illness to any appreciable extent. Transfusions were given at intervals of three to four weeks. Multiple determinations of urobilinogen were made for this patient (chart 4). It is of interest that practically all the preoperative values obtained were well above the upper limit of normal. Values within normal limits were obtained after the first course of liver extract was given parenterally, although there was no improvement in the blood level during this period; furthermore, a second course of parenteral treatment with liver extract exerted no appreciable effect on the abnormally high level of urobilinogen. All that can be said at present of the effect of splenectomy in this case is that the patient seemed distinctly improved; after eight months of hospitalization, the majority of the time being spent in bed, he was able to be up and about and to go home, even though a severe grade of anemia was still present. Two determina-

14. Rhoads, C. P.; Miller, D. K., and Barker, W. H.: Unpublished data.

tions of urobilinogen, made six and seven weeks, respectively, after operation, gave values near the upper limit of normal, suggesting that hemolysis had been reduced.

For 4 patients with normal values for urobilinogen in the feces (cases 25, 44, 45 and 46) the values for urobilinogen in the urine were distinctly above normal, suggestive of hepatic dysfunction. In 3 of the 4 cases there was further evidence of hepatic dysfunction from other tests. In 15 cases of aplastic anemia the urobilinogen value for the urine was either normal or elevated in proportion to the fecal value.

Hepatic function tests were performed on 16 of the 25 patients in this group. Seven patients gave normal results for both the bilirubin excretion test and the hippuric acid synthesis test, 1 patient gave a

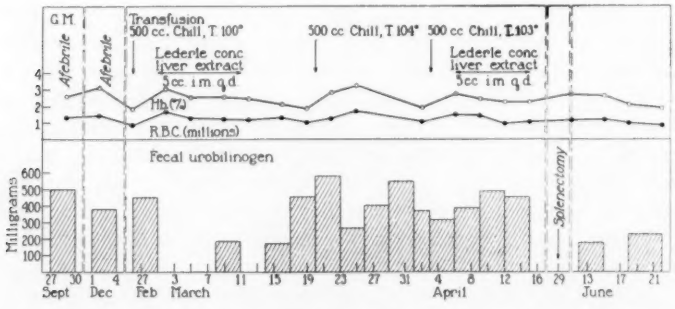
G.M.
Afebrile
Afebrile
Transfusion
500 cc. Chill, T 100°
Lederle conc liver extract 3cc im qd
500 cc Chill, T 104°
500 cc Chill, T 103°
Lederle conc liver extract 3cc im qd
Hb (%)
R.B.C. (millions)
Fecal urobilinogen
Milligrams
Splenectomy
27 30 1 4 27 3 7 11 15 19 23 27 31 4 8 12 16 29 13 17 21
Sept Dec Feb March April June

Chart 4 (case 38).—A man aged 34 with aplastic anemia showed immature cellular marrow. The output of urobilinogen in the feces remained well above normal over a prolonged period, suggesting excessive hemolysis. There was an apparent reduction in the rate of hemolysis after splenectomy.

normal result for the only test performed and 8 showed evidence of hepatic damage. A four-hour bilirubin retention above 5 per cent was present in 6 cases; a four-hour excretion of benzoic acid below 3 Gm., in 4 cases, and evidence of hepatic dysfunction by both tests, in only 2 cases. Thus 50 per cent of the patients on whom hepatic function tests were performed gave evidence of hepatic damage. There does not appear to be a definite association between the type of marrow and the hepatic dysfunction.

Tests of erythrocyte fragility were carried out in 13 of the 25 cases. In 10 cases there was a normal range; in 1, slightly increased fragility (case 31), and in 2, somewhat decreased fragility (cases 36 and 37).

Group 4: Leukemias.—Determinations of urobilinogen in the urine and in the feces were made for 9 patients suffering from various forms of leukemia. The value for the feces was definitely elevated in 2 cases

(cases 47 and 53). It is well to recall here case 21, discussed earlier in connection with the cases of hemolytic anemia. This patient showed severe hemolytic anemia with purpura after exposure to benzene and subsequently died of acute myelogenous leukemia. When he was seen two months before death, the clinical findings and the picture of the bone marrow were those of hemolytic anemia, and proof of leukemia was not at hand. Attention has been directed to the presence of hemolytic anemia in association with leukemia by several authors, among them Jaffé [15] and Beltrametti and his associates.[16] In other cases of leukemia the anemia appears to be due entirely to the replacement of erythroid with myeloid tissue. This was probably the case in the other patients of this group, for whom the output of pigment was either normal (cases 49, 50, 52, 54 and 55) or unusually low (cases 48 and 51).

The urobilinogen content of the urine was elevated in 2 cases (cases 49 and 55), with normal values for the feces, once more suggesting hepatic dysfunction.

The incidence of hepatic dysfunction was high for the group of patients with leukemia; 3 of the 6 patients tested showed abnormal four-hour retention of bilirubin, and 3 of the 4 patients tested showed a four-hour excretion of benzoic acid below 3 Gm.

Group 5: Miscellaneous Conditions.—This group includes patients with several different forms of blood dyscrasia who cannot be included in the four other groups. There were 2 cases of chronic hypochromic microcytic anemia, 5 cases of Banti's syndrome, 2 cases of polycythaemia vera, 6 cases of cirrhosis of the liver and 1 case each of thrombopenic purpura, abdominal Hodgkin's disease with leukopenia, Gaucher's disease and chronic nephritis with uremia.

For 18 of the 19 patients in the group the output of urobilinogen in the feces was determined. In 1 case of Banti's syndrome (case 59) and 1 case of polycythemia (case 64) there was a slight elevation of the urobilinogen in the feces above normal; in the other 16 cases the values were normal or low (case 56 and 60). In case 65 (that of a woman with polycythaemia vera) the output of urobilinogen was studied before and after therapy with phenylhydrazine. The condition proved refractory to the treatment in the dosage used, and there was a relatively slight rise in excretion of pigment, which was reflected in lack of appreciable improvement in the blood picture.

The low value for urobilinogen in the feces in the case of uremia (case 74) suggests that the refractory anemia of chronic nephritis is aplastic rather than hemolytic.

15. Jaffé, R. H.: Erythropoiesis in Leukemia, Folia haemat. **49**:51-63, 1933.
16. Beltrametti, L.; Rettanni, G., and Bascapè, A.: L'anemia nelle leucemie, Haematologica **18**:337-370, 1937.

The average daily excretion of urobilinogen in the urine was determined for 17 of the 19 patients of the group. In 2 cases of Banti's syndrome (case 59 and 62) and in 3 of the 6 cases of hepatic cirrhosis (cases 66, 67 and 69) the excess of urobilinogen in the urine in the presence of normal quantities in the feces may be regarded as evidence of hepatic damage. It is of interest that the quantity in the urine returned to normal after splenectomy in 1 case of Banti's syndrome (case 62).

In only 1 of the 5 cases of Banti's syndrome was there failure to show evidence of hepatic damage by at least one of the two hepatic

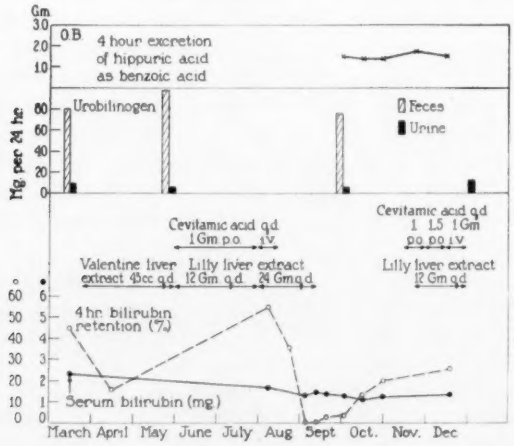

Chart 5 (case 66).—A woman aged 44 had cirrhosis of the liver and macrocytic anemia. The hepatic function and the excretion of urobilinogen were followed for nine months.

function tests employed: in 4 cases there was low excretion of hippuric acid and in 1 case (case 60) marked retention of bilirubin as well. The excretion of hippuric acid rose to normal after splenectomy in 2 cases (cases 58 and 62).

In 5 of the 6 cases of suspected cirrhosis of the liver, evidence of hepatic dysfunction was shown by at least one of the two tests. A four-hour excretion of benzoic acid of only 0.84 Gm. was found in 1 case (case 69), but impaired renal function was probably a factor contributing to this low value.

In another case of cirrhosis of the liver (case 66) the results of repeated hepatic function tests have been charted over a relatively long period, with the urobilinogen values determined at intervals (chart 5). The excretion of benzoic acid remained remarkably constant, between

1.4 and 2 Gm. in four hours; urea clearance was normal in this case, and there was no apparent interference with intestinal absorption, so that the abnormally low excretion of hippuric acid here may be accepted as evidence of poor hepatic function. The four-hour retention of bilirubin fluctuated widely with different tests, from 0 to 55 per cent. Since the serum bilirubin value was constantly elevated above 1 mg. per hundred cubic centimeters, the results are difficult to interpret. The absence of bilirubin retention, as shown by two tests, even though the serum bilirubin value was elevated above 1 mg. per hundred cubic centimeters, is also confusing. Similar results were obtained in certain cases of pernicious anemia previously discussed. It is conceivable that under some conditions the hepatic threshold for serum bilirubin becomes stabilized at a level considerably higher than normal and that the liver varies in its power to excrete an amount of bilirubin raising the serum level above the new threshold. In the case under discussion the urobilinogen in the feces remained quite constant at a low normal level, between 75 and 90 mg. a day. The urobilinogen in the urine was always well above normal, varying from 5.6 to 10.7 mg. a day. This constant elevation of the urobilinogen in the urine, with normal values for the feces, gave additional evidence of severe hepatic damage in this case. Therapy with liver extract and ascorbic acid appeared to benefit the patient considerably, but no objective evidence of improved hepatic function was obtained unless the drop in retention of bilirubin may be accepted as such.

Erythrocyte fragility was studied in 3 cases of Banti's syndrome, 3 cases of cirrhosis of the liver and 1 case of polycythemia. Although the minimum resistance (the point at which hemolysis begins) was normal, the maximum resistance was increased in all 7 cases to between 0.32 and 0.28 per cent.

COMMENT

This study of the excretion of bile pigment in 74 cases of disease of the blood was made to investigate the role of destruction of blood in the production of anemia. Although it has been suggested that bile pigment may be produced through some independent activity of the liver and need not be derived from hemoglobin, no proof for this hypothesis is furnished, and the majority of workers are inclined to accept the theory that the hemoglobin of the blood is the sole source of bile pigment. Except in the presence of hepatic disease or of obstruction to the biliary tract, practically all the bile pigment excreted appears as stercobilin in the feces. The stercobilin can be quantitated with considerable accuracy by Watson's modification of the Terwen method, the result being expressed in terms of urobilinogen. The average daily output of urobilinogen in the feces may then be regarded as an index of the breakdown of hemoglobin or the destruction of blood.

A marked increase in the output of urobilinogen in the feces was the rule for two groups of patients with blood dyscrasias—those with pernicious anemia in relapse and those with hemolytic anemia. The quantity of urobilinogen excreted in a case of severe pernicious anemia in relapse may be just as great as that in a case of congenital hemolytic jaundice. The occurrence of increased destruction of blood in congenital hemolytic jaundice is universally accepted, and it seems as logical to accept excessive hemolysis as an important factor in pernicious anemia. There is no adequate proof as to whether the increased destruction of blood in pernicious anemia is due to the presence of large numbers of abnormal erythrocytes which are readily destroyed by the normal breakdown mechanism of the body or to the activity of a circulating hemolytic toxin.

The observations on pigment metabolism in the aplastic anemias and the hypochromic microcytic anemias furnish further evidence against the nonutilization theory of excessive excretion of pigment in pernicious anemia. Normal or low values for urobilinogen in the feces were obtained in most cases of aplastic anemia and in all cases of hypochromic microcytic anemia studied. In the aplastic anemias, cell formation is obviously inadequate, and in the hypochromic anemias the production of new hemoglobin is depressed. Unless destruction of blood is far below normal in such cases, according to the nonutilization theory, one might expect an output of pigment far above normal, yet the opposite is the case. That a marked decrease in destruction of blood should occur in such cases appears no more likely than the possibility of a marked increase in destruction of blood in pernicious anemia.

Certain other points of interest arose in the course of this study of excretion of urobilinogen. The picture of acute hemolytic anemia has been found to occur occasionally in association with acute leukemia, confirming the observation of previous workers on the subject. Of particular interest were 2 cases of benzene poisoning with hemolytic anemia and purpura; 1 patient made a complete recovery, and the other died of acute myelogenous leukemia. Cabot and Mallory [17] have reported a somewhat similar case of fatal hemolytic anemia, which they attributed to benzene poisoning. Since benzene is fairly well established as one cause of clinical thrombopenic purpura and aplastic anemia, as well as of experimental granulopenia and leukemia, it appears likely that this aromatic compound may cause almost any conceivable type of bone marrow disturbance. If this should prove to be the case, it would bring the hemolytic anemias, the aplastic anemias, the leukemias, the granulopenias and the purpuras much closer together from the etiologic

17. Benzol Poisoning with Bleeding Gums, Hemolytic Anemia, and Active Marrow, Cabot Case 13321, Boston M. & S. J. **197**:236-239, 1927.

standpoint; that is, these various forms of blood dyscrasia might be regarded as different types of response to a single chemical toxin or to closely related aromatic compounds.

The rapid hemolysis of transfused blood, as shown by a sharp rise in the excretion of urobilinogen in the feces after transfusion, deserves further consideration here. This phenomenon probably furnishes an explanation for the failure of many patients with severe anemia to derive more than transient benefit from a transfusion. Also it serves as a warning in interpreting the results of determinations of urobilinogen carried out shortly after a transfusion, for if stools are collected during a period immediately after a transfusion, a value may be obtained that is much higher than the true "basal" value for the particular patient. It is advisable to wait at least ten days to two weeks after a transfusion before starting stool collections for determination of the urobilinogen excretion.

In certain cases of refractory anemia a high urobilinogen value in the feces, serving as an index of increased destruction of blood, may justify splenectomy as a therapeutic measure with the hope of cutting hemolysis down to a minimum and thereby eliminating a hemolytic factor. In 2 such cases splenectomy was followed by sufficient evidence of improvement to warrant further trial in selected cases.

The urobilinogen in the urine may or may not be increased in proportion to the urobilinogen in the feces. The value for the urine is therefore not a safe index of increased excretion of pigment or of increased destruction of blood; it is of more value as an index of hepatic function, for an increased quantity in the urine in the presence of a low or normal amount in the feces may be regarded as conclusive evidence of hepatic dysfunction, as Watson [18] has recently shown.

In addition to the determination of urobilinogen in the urine, two other tests of hepatic function were employed—the bilirubin excretion test and the hippuric acid synthesis test. At least one of the three tests of hepatic function was performed in 63 of the 74 cases, and in 35 of the 63 cases (56 per cent) there was evidence of reduced hepatic function. The incidence is sufficiently high to suggest that a poorly functioning liver may be of etiologic significance in various types of blood dyscrasia. In 24 (71 per cent) of the 34 cases in which all three tests of hepatic function were performed, evidence of hepatic damage was shown by at least one test, the urobilinogen content of the urine was elevated in 10 of the 24 cases, the bilirubin test gave a positive reaction in 13 and the hippuric acid test gave a positive reaction in 16. In only 3 of the 34 cases did all three tests give positive results,

18. Watson, C. J.: Studies on Urobilinogen: III. The Per Diem Excretion of Urobilinogen in the Common Forms of Jaundice and Disease of the Liver, Arch. Int. Med. **59**:206-231 (Feb.) 1937.

but all three tests gave negative results in 10 of the 34 cases. These observations serve to emphasize the importance of carrying out several tests in each case before concluding that hepatic function is normal or abnormal. A single test showing a positive result is highly suggestive of hepatic damage, whereas a single test showing a negative result is of little significance. The possibility that anemia per se with hepatic ischemia is responsible for the impairment of hepatic function seems unlikely in view of the fact that in 7 of the 10 cases in which there was no evidence of hepatic damage by any of the three tests, pronounced anemia was present, with a hemoglobin level of 50 per cent or below.

Since the liver is recognized as the principal detoxifying organ of the body, the high incidence of hepatic damage in various forms of blood dyscrasia suggests that harmful substances inadequately detoxified by an ailing liver may damage the bone marrow and thereby give rise to peculiar refractory anemias or even leukemias.

SUMMARY AND CONCLUSIONS

The excretion of urobilinogen has been studied in 74 cases of blood disease. The average daily output of urobilinogen in the feces is regarded as an index of the rate at which erythrocytes are being destroyed in the body.

Abnormally high values for urobilinogen in the feces were obtained in the cases of hemolytic anemia and in most of the cases of pernicious anemia in relapse; these values returned to normal after splenectomy in the cases of congenital hemolytic jaundice and after adequate liver therapy in the cases of pernicious anemia.

Based on these observations, the conclusion is reached that destruction of blood is increased in pernicious anemia, even though the disease may not be primarily a hemolytic anemia.

The anemia occurring in association with leukemia is occasionally hemolytic. In rare cases of aplastic anemia a hemolytic factor is apparently active; splenectomy in 2 such cases was followed by temporary improvement.

Transfusion of well matched blood may at times be followed by a striking increase in the excretion of bile pigment, suggesting that the better part of the transfused blood is rapidly hemolyzed in such instances.

In 63 of the 74 cases of blood disease the hepatic function was studied by one or more of three tests: the test for urobilinogen in the urine, the bilirubin excretion test and the hippuric acid synthesis test. Evidence of hepatic dysfunction was obtained in over 50 per cent of the cases, suggesting that a disturbance in the function of the liver may play a significant role in the pathogenesis of many of the blood dyscrasias.

EXPERIMENTAL STREPTOCOCCIC ENDOCARDITIS

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AND
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ST. LOUIS

I. REPRODUCTION OF THE DISEASE BY MEANS OF INTRAVENOUSLY INJECTED STREPTOCOCCI

The fact that the treatment of bacterial endocarditis remains unsuccessful emphasizes the difficulty involved in understanding this disease. The utter failure to treat the disease successfully with such immunologic agents as serums and vaccines indicates also that the factors at work within the animal body are distinctive and for the most part unidentified. In a general way, two facts seem to be established by clinical studies: first, the patient who acquires bacterial endocarditis has invariably suffered previous injury to the tissues of the valves, and, second, bacterial endocarditis is established by the implantation in or on this injured valve of bacteria, usually nonhemolytic streptococci, which gain access to the blood stream through the medium of a local infection such as pharyngitis or otitis media. In some instances it is not known that previous injury to a valve existed. It is possible that an acute erosive injury to the valve received during an acute infection such as pneumonia may be followed promptly by implantation of bacteria. Recently reported studies of Nedzel [1] revealed the effect of intravenous injections of pitressin on the integrity of the endothelium of the mitral valve. Furthermore, it may be necessary to suppose that an old injury, rheumatic in orgin, must be awakened by the intercurrent local infection in order for the valve to be susceptible to implantation. Again, there are certain instances in which the presence of an alleged intercurrent local infection cannot be discovered by physical examination or by study of the history and the route of infection remains undetermined. How the influence of exposure to cold operates is not clear, but such exposure may be the only feature of the history. The possibility of infection through the intestinal tract is by no means fanciful.

The mere recital of these features indicates how difficult it is even to state the question when approaching the discussion of this disease.

From the Department of Internal Medicine, St. Louis University School of Medicine.

1. Nedzel, A. J.: Experimental Endocarditis, Arch. Path. **24**:143 (Aug.) 1937.

The present report deals with an attempt to approach the problem, first, by reproducing the disease in dogs and then by studying the effect of experimental treatment.

METHOD

Dogs weighing about 10 Kg. each were used. These animals are sufficiently robust to tolerate the experimental conditions and possess carotid arteries large enough to admit the instrument used to injure the valves.

The instrument used is a plated rod, about 2 mm. in diameter, which is tapered slightly and terminates at one end in a hook the inner border of which is beveled on both sides to give a cutting edge. The hook is compressed in its transverse diameter so as not to increase materially the width of the instrument at the point where it enters the artery. A suitable handle fits the other end of the rod. The length of the rod is about 18 inches (46 cm.). These measurements are influenced by the length and the size of the lumen of the carotid artery.

The neck was shaved, and tincture of iodine was used to cover the site of operation, over the right carotid artery. After anesthesia was established with ether or amytal, an incision was made over the line of the right common carotid artery. Appropriate dissection exposed the carotid sheath. The artery was clearly isolated for 2 or 3 inches (5 to 8 cm.) of its length, and two ligatures of linen were slipped beneath the artery. The distal ligature was tied. When the artery was lifted with the left index finger, the vessel could be compressed and a small cut could be made through the wall with a sharp scissors. Pressure by thumb and forefinger stopped the loss of blood, and the hooked end of the instrument was inserted through the cut and the rod was slid down into the aortic orifice of the heart. With practice it was possible to acquire the ability of perforating an aortic cusp. When the instrument was withdrawn, the cutting edge of the hook severed the cusp. If the aortic valve was not engaged, it was easy to hook the edges of the nearby mitral leaflet. The establishment of suitable injury was determined by the production of a loud murmur that persisted after the hook was withdrawn into the carotid artery. The proximal ligature was then tied, and the wound was closed.

Infection of the injured valve was attempted by the inoculation intravenously of a twenty-four hour plain broth culture of nonhemolytic streptococci of little or no virulence for white mice. It was considered important to wait at least thirty days after the operation before injecting these bacteria.

Culture of the blood was made by drawing blood from the left jugular vein. Agar was considered to be the best medium, since it permits the more definite identification of contaminating bacteria. Measured amounts of blood were used in order to determine the number of colonies. In case of doubt, the identity of colonies in relation to the strain injected was established by comparing fermentation reactions in the usual mediums containing carbohydrates.

RESULTS

In a previous report [2] the production of streptococcic endocarditis was described. At the time of that report the animals studied lived from twelve to eighteen days after infection of the injured valve. The period was too short for the study of therapeutic agents, even though

2. Kinsella, R. A.: Proc. Soc. Exper. Biol. & Med. **20**:252, 1923.

all the salient features of the disease in human beings were reproduced. Also, the conditions of the experimental infection were too severe to permit measured study. The chief modifications in procedure which led to more favorable results were the employment of streptococci of low virulence and the infection of the animals after allowing at least thirty days to elapse after the operation. With our present method, healthy dogs will tolerate the operation, remain in good condition until infected and then withstand the experimental disease for twenty-five days. Occasionally an animal lives for more than thirty-five days. Successfull implantation of the cultures is determined by obtaining positive results of blood culture repeatedly.

Abortive Infection.—This has occurred not infrequently, as demonstrated by the recovery of streptococci in small numbers, viz., two to five colonies per cubic centimeter of blood, followed by repeatedly sterile cultures with the animal remaining healthy. This curious occurrence may have its representation in human beings with acute rheumatic endocarditis for whom one or more positive results of blood culture are obtained without the establishment of bacterial endocarditis. Whether or not in such cases there are colonies of bacteria implanted on the surfaces of the injured valves without the production of underlying reaction was not determined except for those dogs which were treated by the method to be described later in this paper; nor was it possible to determine why such transitory bacteremia occurs without complete development of bacterial endocarditis. The animals in such cases are not as definitely sick as they are when the bacteremia becomes continuous, nor do they display the peripheral embolic effects of the fully developed infection.

Death Rate.—No animal was ever found to have recovered from the definitely established infection of a cardiac valve. Eighty-seven dogs were studied in which increasingly severe illness characterized by embolic episodes attended the continuous bacteremia.

Bacteremia.—Figure 1 presents the curve for colony counts in typical fatal bacteremia. The bacteremia was uniformly of the increasing type, the colonies beginning in low concentration and ending in countless numbers. The shorter the duration of life, the more acute the angle of ascent of the curve representing the colony counts. This is unlike the appearance of the chart in cases of bacteremia in human beings. In the latter case the colony count is maintained at a uniform level, either high or low, throughout life.

Suppressed Bacteremia.—It seems likely that whatever factor controls the bacteremia, keeping the number of colonies per cubic centimeter of blood at a constant level, is associated with protection. In order to inquire into the question of whether or not immunization to streptococci is responsible for control of bacteremia, experiments were carried out:

A. In the first series of these experiments, 6 animals were inoculated two weeks after valvotomy with cultures of living streptococci, previously exposed for thirty minutes at 37 C. to the action of serum of dogs that had died of the infection. This serum caused the organisms to become agglutinated. Four of these 6 animals displayed a tendency to constancy of the colony count (figure 2 illustrates this result). As other animals at a later date, surviving for thirty to thirty-

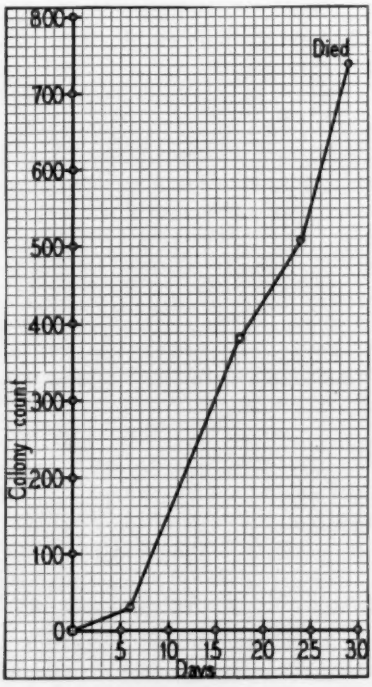

Fig. 1.—Bacteremia in a dog that had not been treated. The colony count indicates the number of colonies per cubic centimeter of blood.

five days, also displayed this tendency without being infected by such agglutinated bacteria, it is unsafe to conclude that the agglutinins so injected conveyed any influence responsible for the suppressed bacteremia.

B. In a second series of experiments, 2 dogs previously injured by valvotomy received intravenous injections of killed cultures of nonhemolytic streptococci every four days until five such injections had

been made. At this time the serum of both animals gave strong agglutination reactions with the cultures used for injection. Subsequent injections of living cultures produced the usual fatal disease.

C. In a third series of experiments, 4 animals previously injured by valvotomy received injections of living hemolytic streptococci in one knee joint. Although local inflammatory reaction developed, culture of the blood remained sterile in each instance for ten days. One animal was killed and showed a traumatized cardiac valve without infection. The other 3 animals were successfully infected by intravenous injection and at autopsy displayed typical bacterial vegetation.

Pathologic Features.—The accompanying photographs illustrate effectively the complete similarity between the experimental infection and the disease in human beings.

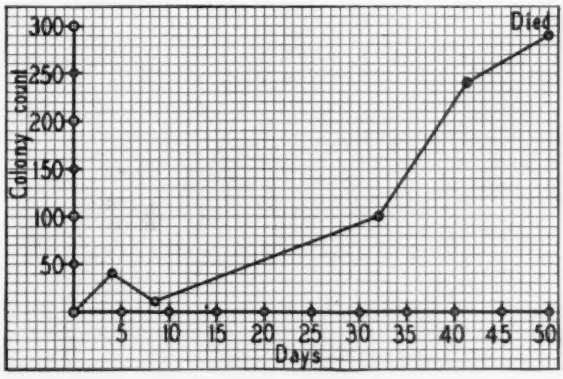

Fig. 2.—Bacteremia produced by the use of agglutinated organisms.

The vegetations (fig. 3) were of varying sizes and showed a capacity to spread infection by contact. Thus a vegetation originating on an injury of one of the aortic cusps would apparently cause a vegetation to develop on the nearby leaflet of the mitral valve. Even when no gross evidence of vegetation could be seen, inspection of the tissue under the microscope revealed spreading bacterial growth along the surface of the valve, onto the auricular surfaces and along the surfaces of the chordae tendineae, often without any underlying cellular response.

Microscopically (fig. 4) the vegetation consisted of dense masses of bacteria, loosely held in a matrix of pink-staining material, with rich leukocytic infiltration of the underlying valvular structures.

Typical embolic localizations occurred in the kidney and spleen. Cerebral infarction was not infrequent. More interesting were the

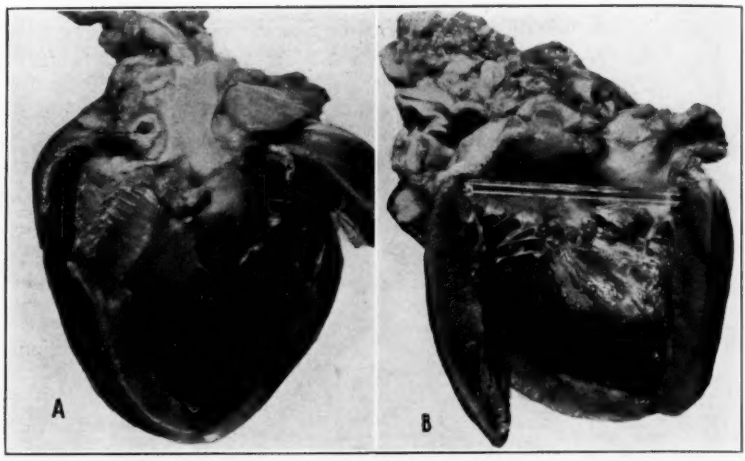

Fig. 3.—*A* shows vegetation on the aortic cusp; *B* shows vegetation on the mitral valve.

Fig. 4.—Microscopic study of the vegetation on the mitral valve, showing colonies in a pink-staining matrix, with an underlying zone of neutrophils and lymphocytes.

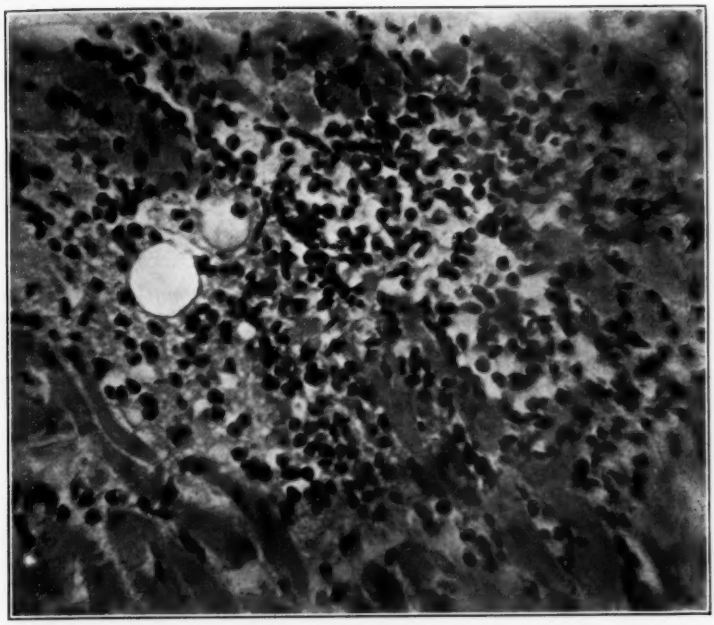

Fig. 5.—Photomicrograph showing punctate hemorrhage in the cardiac muscle at the stage when infiltration with neutrophils and lymphocytes had begun.

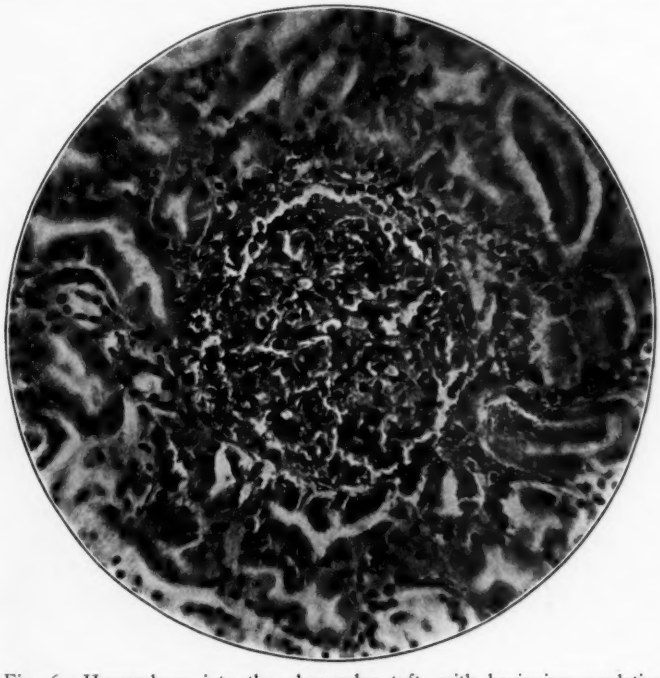

Fig. 6.—Hemorrhage into the glomerular tuft, with beginning exudation of neutrophils and lymphocytes.

minute vascular lesions which were present in the brain, heart (fig. 5), skeletal muscles and glomeruli (fig. 6). These lesions seemed to begin as punctate hemorrhages which became infiltrated with leukocytes and finally with small round cells. The animals did not show any later development of the lesions. These lesions could easily account for the disturbances sometimes manifest in the electrocardiogram, probably for the mental changes in human beings, for the appearance of red and white blood cells in the urine, for the petechiae which appeared in the conjunctiva and in the skin of some of the animals and for the spotted appearance of the surface of the kidneys (fig. 7). These lesions have

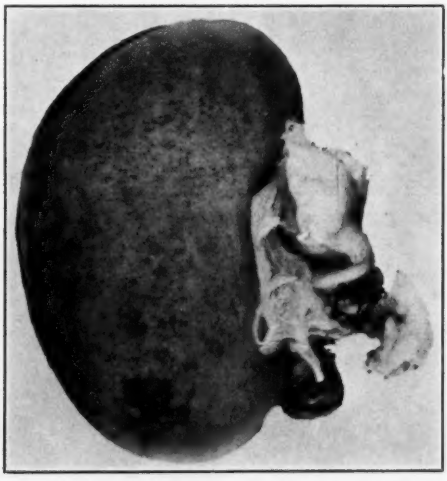

Fig. 7.—Photograph showing the typical "flea-bitten" kidney.

often been assumed to be of embolic formation. Serial sections made through a petechial lesion in the skin failed to throw light on their mode of formation.

COMMENT

Bacterial endocarditis can be produced in dogs by the methods described. This disease in dogs presents features identical with those observed in the disease in human beings. The difference in the bacteremia is quantitative and no doubt depends on the absence of those factors which make the duration of the disease in human beings more prolonged. Of particular interest are the lesions in the kidneys—the glomerular thromboses (fig. 8), so characteristic of the disease in human beings. The fatal character is invariable in dogs—as it seems to be in

human beings. Variations occur, implantation of bacteria being defeated either because of failure of the bacteria themselves to gain a foothold or because of some indifference on the part of the tissues. The evidence obtained does not support the idea that animals can be immunized either by intravenous inoculation of killed streptococci or by the establishment of a local infection. Nor were we able to bring about infection of the injured valve through the medium of such a local infection.

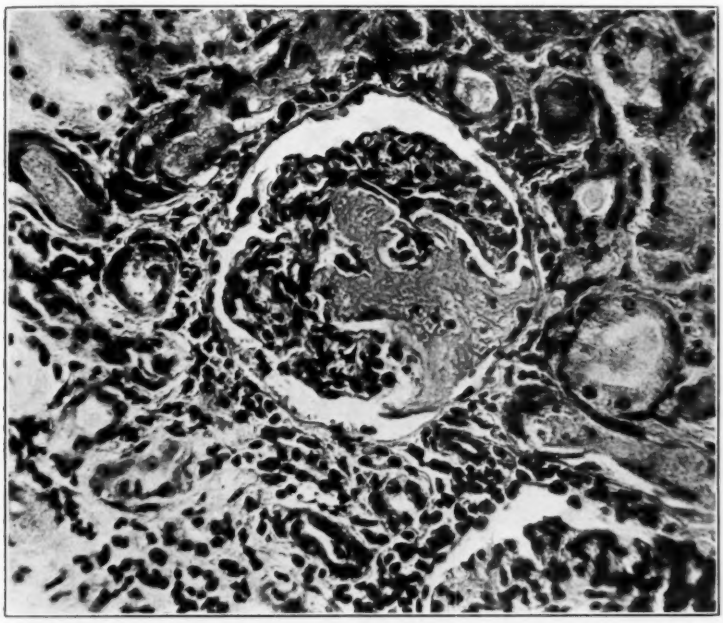

Fig. 8.—Photomicrograph showing thrombosis of part of a glomerular tuft.

The assumption is invited that infection of the valve in human beings, if it does occur through the medium of local infection, is further encouraged by changes going on in the injured valve itself which make implantation possible. According to this reasoning, an old rheumatic lesion of the valve does not become converted into bacterial endocarditis with any and every intercurrent bacteremia which may occur during the life of the individual but requires some change in its own economy to bring about such a disaster.

II. TREATMENT OF THE DISEASE

Many attempts have been made to effect a cure in patients suffering from bacterial endocarditis. Perhaps the literature does not contain a description of the most bizarre of these attempts. Serums, variously produced, and vaccines are some of the agents employed. These agents are so uniformly unsuccessful that the inference might be drawn that the immunologic approach to treatment cannot succeed.

This part of our report deals with the study of the effect of treating the experimental disease by intravenous injection of an organic mercurial drug (merthiolate) and by oral administration of sulfanilamide.

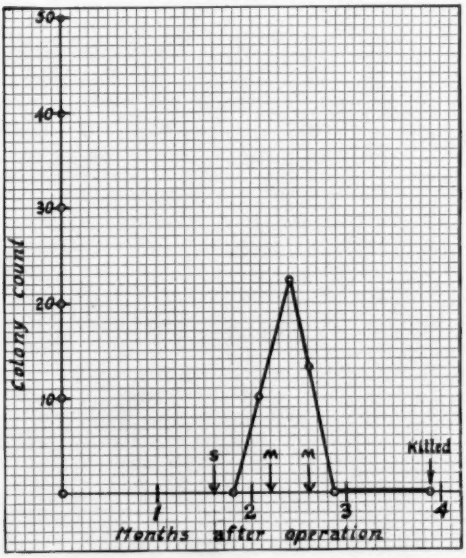

Fig. 9.—Bacteremia lasting for eighty-six days. In charts 3 to 7, *S* indicates intravenous injection of 10 cc. of a culture of streptococci; *M*, injection of 10 cc. of 1 per cent solution of merthiolate.

TREATMENT WITH MERTHIOLATE

Method.—Animals were prepared by the method previously described. Care was taken to select dogs weighing from 10 to 12 Kg. One month was allowed to elapse after the operation before streptococci were injected intravenously. When the resulting cultures yielded only a few colonies per cubic centimeter of blood, several positive results of blood culture were obtained before merthiolate was injected. In this way the possibility of an "abortive infection" was excluded. When bacteremia was established, with a high colony count, merthiolate was used without waiting for further positive results of blood culture.

Buffered merthiolate was used in most instances in a dosage of 1 cc. of 1:100 dilution to each kilogram of body weight of the dog. When a dilution of 1:1,000 was used, less effect was noted. The diluent was isotonic buffered salt solution.

For this part of the study there were 14 animals from which acceptable evidence could be obtained. Other animals were excluded on account of accidents which interfered with the experiment.

Clinical Results.—During the attempts to treat animals with merthiolate, it was found to be especially true that strains of streptococci differ in their capacity to produce bacteremia. Thus the strain which was called CH immediately produced infection, with counts of over

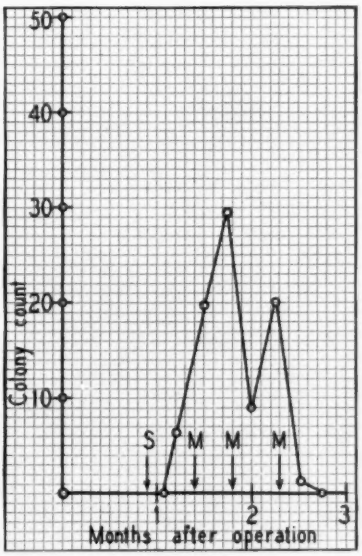

Fig. 10.—Bacteremia lasting for seventy-five days. This animal was killed eight and one-third months after operation. Culture of the blood at autopsy was sterile.

1,000 colonies per cubic centimeter of blood. Against this type of bacteremia, merthiolate was scarcely effective, producing only slight reductions in the colony count. There were 5 animals in this group.

A strain called G, which was unfortunately lost after a short time, produced a type of bacteremia characterized by a small number of colonies per cubic centimeter of blood. This type of infection yielded readily to intravenous injections of merthiolate. There were only 2 animals in this group, and both were apparently completely cured (figs. 9 and 10). One of these animals was killed forty-four days

after being infected and twenty-three days after the last positive result of blood culture. The other animal was allowed to live for eight months after the last positive result of blood culture.

Seven of the animals studied were given intravenous injections of cultures of a strain of streptococci called WK. This strain seemed to stand midway between G and CH as to virulence for dogs and produced a type of bacteremia which rapidly increased in severity and tended to recur even after successful sterilization with merthiolate.

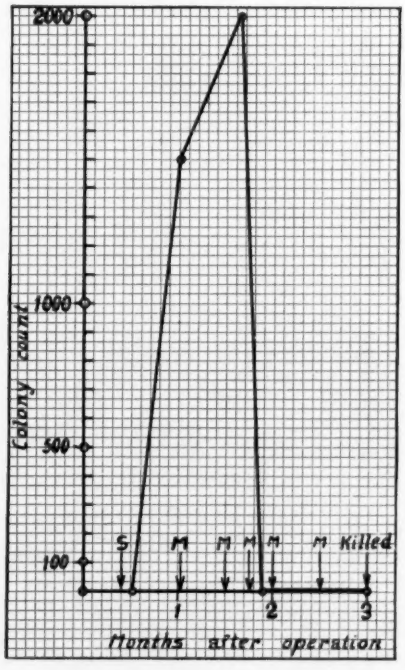

Fig. 11.—Bacteremia lasting for thirty days. The animal was killed after three months; culture of the blood was sterile.

Three animals in this series (figs. 11 and 12) were apparenly cured. In 1 of these animals (fig. 11) bacterial masses were seen microscopically on the surface of the valve, without underlying cellular response (fig. 13). Although cultures of the heart blood made at autopsy were sterile, the tissue of the valve was not ground for culture. It is therefore impossible to state whether the bacteria were viable. This interesting observation will be discussed later.

In 1 case (fig. 14) there was an apparent cure, although the animal died of pneumonia before observations were completed. This animal likewise displayed bacterial masses without underlying reaction (fig. 4).

One animal (fig. 15) was treated without cure but with prolongation of life and with a definite effect on the severity of the bacteremia. The

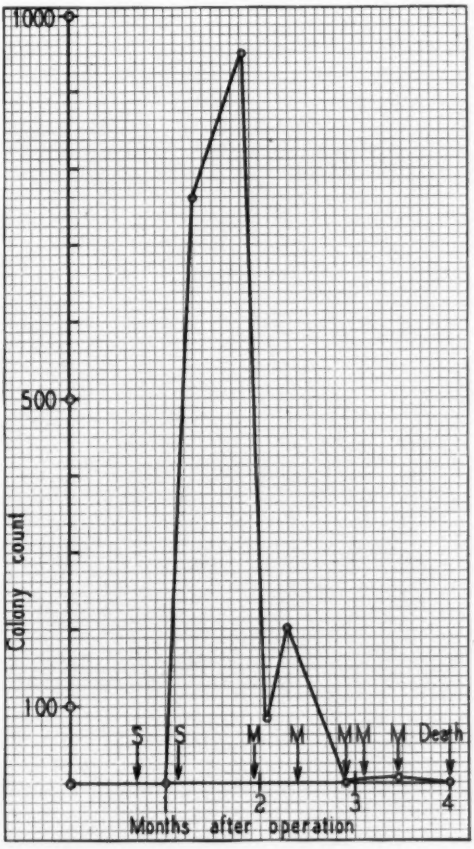

Fig. 12.—Bacteremia lasting for seventy-three days. At autopsy, culture of the blood was sterile.

apparent explanation of the failure of the drug in this case is to be found in the fact that insufficient amounts of merthiolate were employed.

The last 2 animals in this series which were given injections of strain WK were incompletely observed because death intervened (in

1 case due to cerebral infarction and in the other to pneumonia) before the effects of merthiolate (already favorable) could be established.

Of the 9 animals given injections of strains G and WK, 5 were clinically cured, 2 showed remarkable improvement and 2 were incompletely observed because of early death.

Effect of Successful Treatment on Reaction of the Tissues of the Valves.—As has already been shown (fig. 3), the disease established on the injured valve presents gross and microscopic features identical with those observed in human beings. The vegetations consist of

Fig. 13.—Photomicrograph showing the remains (?) of heavy bacterial vegetation in a cured animal. Note the absence of signs of underlying reaction in the tissue of the valve.

masses of bacteria encrusted on the surface of the valve and neighboring endocardium. Immediately underneath these bacterial masses is a dense infiltration of leukocytes, occupying most of the thickness of the valve. In the cases of 2 animals (figs. 9 and 12), both of which had apparently recovered from infection and which yielded sterile blood culture at autopsy, masses of bacteria, perhaps dead, occupied the surface of the valve, but there was no underlying reaction in the tissues. It is unfortunate that such valves were not ground and cultures made

to determine the viability of the bacteria in question. It can probably be assumed that these bacteria were not viable. That they did not break away from the surface of the valve and cause embolic effects seems remarkable. Analogy is suggested with the condition of the valves observed in some cases of acute rheumatic fever in human beings recently discussed by Von Glahn and Pappenheimer.[3] These patients showed transiently positive results of blood culture and at autopsy the

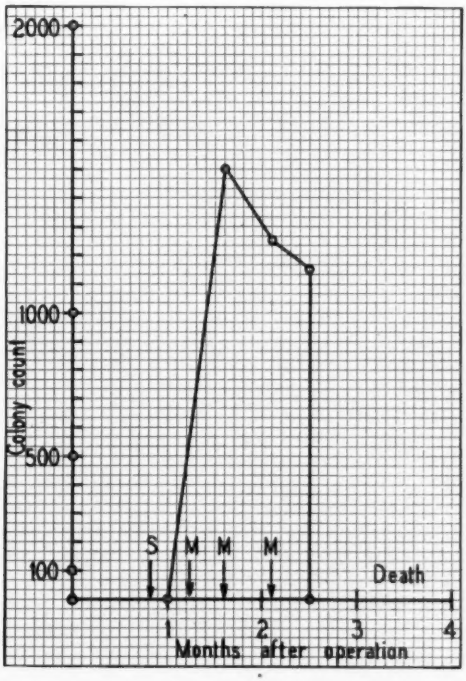

Fig. 14.—Bacteremia lasting for thirty-two days. At autopsy the culture of the blood was sterile.

valves were studded with microscopic colonies of streptococci, without underlying reaction in the tissues. The evidence presented by the study of experimental bacterial endocarditis in dogs suggests no relation between acute rheumatic fever and bacterial endocarditis. As a matter of fact, the two diseases are never seen in human beings simultaneously. The

3. Von Glahn, W. C., and Pappenheimer, A. M.: Relationship Between Rheumatic and Subacute Bacterial Endocarditis, Arch. Int. Med. **55**:173 (Feb.) 1935.

question is left open as to whether merthiolate kills the bacteria directly or renders the tissues underlying the implanted masses impervious or indifferent.

Appearance of the Healed Valve.—In those animals which were cured and which came to autopsy between six and eleven months after sterile culture of the blood was obtained continuously, the microscopic appearance was that of glistening verrucae, coarser than those seen in

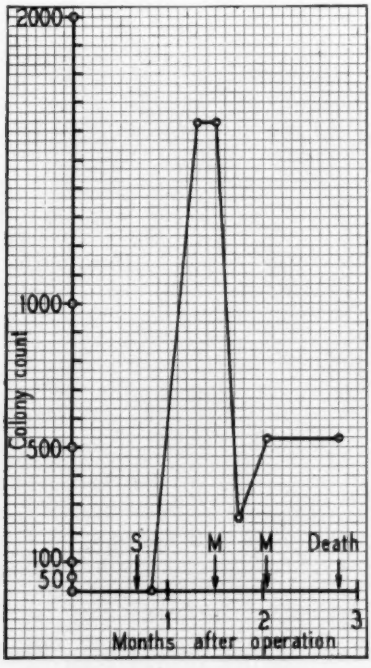

Fig. 15.—Bacteremia lasting for thirty-nine days. *S* indicates injection of 10 cc. of a culture of streptococci. The first injection of merthiolate (1:1,000), *M*, consisted of 1 cc.; the second, of 2 cc.

human beings with old rheumatic disease. Microscopically (fig. 16 *A*) there was a granulomatous arrangement, with frequent large but not definitely polynuclear cells—a picture sometimes, but most probably incorrectly, likened to that of Aschoff bodies. A comparison of such a picture with that of the valve which has been injured and has healed without infection (fig. 16 *B*) is interesting.

It seems to be logical to compare the healed infected valve with the healed rheumatic valve of a human being. Further studies are projected in which such animals will be reinfected and a repetition of the therapeutic attempts will be carried out.

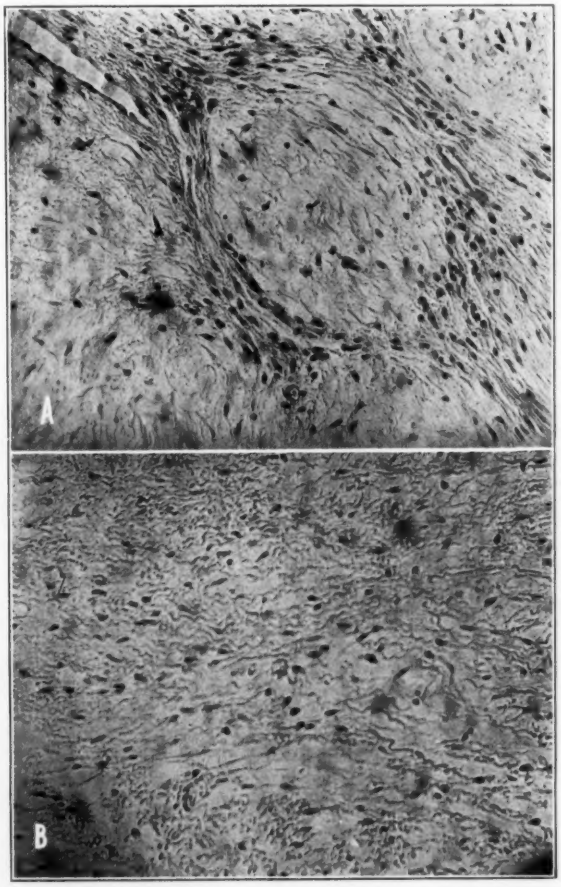

Fig. 16.—*A*, cross section of a valve that had healed after the infection; *B*, cross section of a valve that had healed after trauma without infection.

The failure of merthiolate and sulfanilamide to be useful in the treatment of human beings with streptococcic endocarditis and their success in the treatment of dogs may depend on the rather fundamental difference between the infected traumatized valve in the dog and the infected diseased valve in the human being.

TREATMENT WITH SULFANILAMIDE

The successful treatment of streptococcic infections with sulfanilamide (para-aminobenzenesulfonamide) by Long and Bliss [4] in this country, after the investigations of Domagk [5] and others in Europe, has given emphasis to the study of chemotherapy in infectious diseases. We have had success in the treatment of experimental endocarditis with merthiolate. We have not, however, been able to apply this therapy successfully to endocarditis in human beings and therefore have continued to search for a more satisfactory drug. Sulfanilamide was chosen for further investigation since its applicability to human beings was already proved. Furthermore, it was deemed wise to note the effects of the drug in the experimental animal before giving it to the patient suffering from subacute bacterial endocarditis.

Method.—Five dogs weighing 10 Kg. each were anesthetized by the administration of a barbiturate into a vein. The carotid artery was exposed, and the hooked instrument was passed down into the heart. An attempt was made to injure a valvular leaflet; if a murmur persisted after withdrawal of the instrument, success was indicated.

All the animals had persisting murmurs after recovery from the operation. About two weeks after the operation each dog except dog 137 was given 10 cc. of a culture of Streptococcus viridans intravenously. Dog 137 was fed Str. viridans for thirty-three days before the organisms were given intravenously. Blood was taken daily for culture, and when culture gave positive results on three successive days the use of the drug was started. Two dogs (dogs 237 and 937) failed to yield a positive result of culture and required a second injection.

Once the infection was established, treatment with sulfanilamide was started. The dose was arbitrarily set at 20 grains (1.3 Gm.) per day, and each dog received the daily dose through a stomach tube. Blood was taken for culture every two or three days, and treatment with the drug was continued until the dog had been free from bacteria for from three to five days. Once the use of the drug was stopped, culture of the blood was repeated at less frequent intervals. In one instance (dog 937) treatment was continued for thirty-eight days because the results of culture remained positive. The dog finally died, apparently of the infection.

Results.—The five dogs gave negative results of blood culture five and ten days after operation. Each dog showed a positive result after intravenous injection of living streptococci; 2 dogs required a second injection. Once a positive result of culture was obtained, the bacteria were constantly present, usually in increasing numbers, until therapy was introduced.

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4. Long, P. H., and Bliss, E. A.: Para-Amino-Benzene-Sulfonamide and Its Derivatives: Experimental and Clinical Observations on Their Use in the Treatment of Beta-Hemolytic Streptococcic Infection; Preliminary Report, J. A. M. A. **108**:32 (Jan. 2) 1937.

5. Domagk, G.: Klin. Wchnschr. **15**:1585 (Oct. 31) 1936.

Twenty grains (1.3 Gm.) of sulfanilamide given daily successfully freed the blood stream of 4 dogs of bacteria. One dog died despite treatment, although the course of the septicemia was prolonged to thirty-eight days (fig. 3 *A*).

The drug in the doses employed did not seem to be toxic for any dog. The 4 survivors were well after periods ranging from three to six months.

The examination of the tissues of dog 937 showed evidence of severe septicemia. Sulfanilamide could not be held responsible for this death.

Conclusion.—On the basis of our work we must conclude that sulfanilamide in the doses used is an effective agent for freeing the blood stream of Str. viridans.

Four dogs were cured of persistent septicemia with Str. viridans by the administration of sulfanilamide. One dog succumbed to the infection, although the drug was administered in the usual way.

III. REPRODUCTION OF THE DISEASE BY MEANS OF ORALLY ADMINISTERED STREPTOCOCCI

The production of an infectious disease by the oral administration of bacteria has been accomplished in the case of typhoid by Metchnikoff and Bezredka.[6] One may be critical of the similarity which this experimentally produced infection bears to what might be called the typical clinical picture of typhoid. A review of the literature does not reveal any other work in which a clinical infection as seen in man has been reproduced in animals by the feeding of the etiologic agent.

On the other hand, numerous workers have reported successful immunization of animals by feeding living and killed bacteria. Most of this work has been carried out with typhoid and paratyphoid and dysentery bacilli.

During our study of streptococcic endocarditis produced by injuring the cardiac valves of dogs with subsequent intravenous inoculation with streptococci, an attempt was made to study the possibility of protecting animals after injury of the valves by feeding killed and living streptococci. While a number of such animals were unaffected by the feeding and later became infected by intravenous inoculation, several animals became sick before intravenous inoculation, and at autopsy were observed to have vegetative lesions on the injured valves. On microscopic examination these vegetations were seen to be composed of streptococci.

6. Metchnikoff, I., and Bezredka, A.: Terap. Obozr. **4**:161, 1911; Ann. Inst. Pasteur **25**:193, 1911.

After this fortuitous result, a deliberate attempt was made to study the possibility of infecting injured cardiac valves by feeding streptococci.

METHODS

Animals were selected which weighed about 10 Kg. and were free from infection after a suitable period of isolation. The operation was performed with the animal under amytal narcosis. Usually it resulted in cutting the cords of the mitral valve. This injury had proved sufficient for the implantation of subsequently injected bacteria.

For the first series of animals, 10 cc. of pure twenty-four hour plain broth culture was mixed with food and presented to the animals. For the second series of animals the culture was administered through a small stomach tube.

TABLE 1 *(Group 1).—Positive Results*

Dog	Operation	Feeding	First Positive Blood Culture	Death
4a	11/25/25	11/27	12/ 2	12/ 4
7	12/17/35	12/18	12/24	12/29
9	12/17/35	12/18	12/24	12/26
4	12/ 4/35	12/ 6	12/ 9	1/21
15	4/ 1/35	4/ 2	4/11	4/12
22	3/24/35	4/ 6	4/ 9	4/10

TABLE 2 *(Group 1).—Negative Results*

Dog	Operation	Feeding	Feeding Stopped	Number of Blood Cultures	Intravenous Inoculation	First Positive Blood Culture	Death
13	1/13/36	1/14	2/25	10	2/25	2/29	3/ 1
12	1/ 8/36	1/10	2/12	8	2/12	2/16	2/19
14	1/20/36	1/21	2/12	5	2/12	2/19	....
10	12/30/35	1/ 1	1/ 7	3	1/ 7	1/13	....
6	12/25/35	12/26	1/ 3	5	1/ 3	1/ 9	3/16

Blood culture was made every three or four days during the experiment. When vegetations were observed at autopsy, the bacteria of the vegetations as well as the bacteria found in blood cultures were studied to determine their identity. To study the identity, the agglutinability of the recovered bacteria with antiserum homologous with that of the injected bacteria was tested. Also the fermentation reactions were compared, inulin, sucrose, mannitol and lactose being used as test substances.

RESULTS

In the first series of dogs, which were given the streptococci in broth culture mixed with the food, there were 11 animals. Tables 1 and 2 show the interval between the operation and the date of the beginning of feeding with streptococci. Six animals became infected, as shown by positive results of blood culture, and 5 animals, although fed as soon after the operation as the others, did not become infected. After

from three to ten sterile cultures were obtained, these animals were inoculated intravenously with streptococci, and endocarditis developed. This last fact indicates that the injury to the valve in these animals was sufficient to permit the implantation of bacteria.

In those dogs which became infected promptly after the feeding with streptococci was begun, the infection was of the fulminating type, such as is seen in animals inoculated intravenously within the week after injury of the valves.

In those dogs which did not become infected by the feeding, no immunity was developed by the feeding. In the case of dogs 12 and 13, the intravenous injection of streptococci occurred thirty-five and forty-three days, respectively, after the operative injury. This should have led to the more prolonged disease, according to extensive previous

TABLE 3 *(Group 2).—Positive Results*

Dog	Operation	Feeding	First Positive Culture	Result
137	2/ 2/37	2/ 4	2/ 6	2/ 9 Death
937	3/25/37	3/26	3/31	4/15 Treatment
737	3/25/37	3/26	3/31	4/15 Treatment
837	3/25/37	3/26	4/ 2	4/16 Treatment

TABLE 4 *(Group 2).—Negative Results*

Dog	Operation	Feeding	Feeding Stopped	Bacteria Injected	First Positive Culture	Result
337	2/5/37	2/6	2/22	2/26	3/1	3/8 Death
237	2/2/37	2/4	2/16	2/17	3/1	3/5 Treatment

experience. However, it will be seen that all the dogs in this group lived only a short period (four, four, seven, six, six days, respectively). In the case of dogs 12 and 13, it is suggested that the endocarditis may have existed before the intravenous inoculation was made, thus adding to the number of animals infected by feeding

In the second series of animals the culture of streptococci was put in the stomach through a small stomach tube. Four of the 6 animals in this series became infected, as revealed by repeatedly positive results of blood culture. The 2 animals which showed sterile blood cultures after what seemed an adequate period of feeding were then inoculated intravenously with streptococci and on being infected were utilized for the study of the treatment with drugs.

Tables 3 and 4 show the results in this series.

Bacteriology.—The strains of streptococci obtained by culture of the blood and by culture of the vegetations removed at autopsy were com-

TABLE 5 *(Group 1).—Sugar Reactions**

Sugars	Dog from Which Bacteria Were Obtained							
	4a	4	10	6	9	7	8743	8911
Mannitol	—	A	A	A	A	A	A	A
Lactose	A	A	A	A	A	A	A	A
Sucrose	A	A	A	A	A	A	A	A
Melitose	—	—	—	—	—	—	—	—
Inulin	—	—	—	—	—	—	—	—
Salicin	A	A	A	A	A	Slight A	A	A

* The sugar reactions were read at the end of 48 hours, at which time they had become constant. A indicates acid reaction. Bacteria obtained from a patient were used in this experiment.

TABLE 6.—*Agglutination Test*

Dilution	Dog from Which Bacteria Were Obtained							
	4a	4	10	6	9	7	8743	8911
1:10	4+	4+	4+	4+	4+	4+	4+	4+
1:40	4+	4+	4+	4+	4+	4+	4+	4+
1:80	4+	4+	4+	4+	4+	4+	4+	4+
1:160	4+	4+	4+	4+	4+	4+	4+	4+
1:320	4+	4+	4+	4+	4+	4+	4+	4+
1:640	4+	4+	4+	4+	4+	4+	4+	4+
1:1,280	4+	4+	4+	4+	4+	4+	4+	4+
Control serum	—	—	—	—	—	—	—	—
Organisms	—	—	—	—	—	—	—	—

TABLE 7 *(Group 2).—Fermentation Tests**

Sugars	Dog				
	2B37	8B37	7B37	9B37	Control
Mannitol	A	A	A	A	A
Lactose	A	A	A	A	A
Sucrose	A	A	A	A	A
Melitose	—	—	—	—	—
Inulin	—	—	—	—	—
Salicin	Slight A	A	A	Slight A	A

* The cultures were read after forty-eight hours.

TABLE 8 *(Group 2).—Agglutination Reaction*

Dilution	Dog			
	2B37	8B37	7B37	9B37
1:10	4+	4+	4+	4+
1:40	4+	4+	4+	4+
1:80	4+	4+	4+	4+
1:160	4+	4+	4+	4+
1:320	4+	4+	4+	4+
1:640	4+	3+	4+	4+
1:1,280	4+	3+	3+	4+
Normal serum	0	0	0	0
Control organism	0	0	0	0

pared with the strain used in the feeding. Tables 5 to 8 show the complete identity of these strains as regards both agglutinability and fermentation reactions.

Pathologic Data.—Grossly the vegetations on the mitral valve and along the injured chordae tendineae were poorly developed though definite. They did not attain the size of vegetations usually seen in animals inoculated intravenously thirty days or more after operative injury. They were like the vegetations seen in animals inoculated intravenously a few days after such injury. In other words, since the endocarditis was of short duration, the vegetations were not large. Besides the endocardial lesions, infarcts of typical variety were present in the spleen, kidney and elsewhere. Microscopically the vegetations were composed of masses of streptococci.

SUMMARY

Seventeen dogs were subjected to operation whereby the mitral valve or the chordae tendineae were cut. All these animals were then fed with living cultures of nonhemolytic streptococci either mixed with food or by stomach tube. Ten of the animals became sick, displayed positive results of blood culture and died. At autopsy these infected animals had bacterial endocarditis. The bacteria in the vegetations were determined to be identical with those that had been fed to the animals.

CONCLUSIONS

Streptococcic endocarditis can be produced in dogs with injured cardiac valves by feeding them streptococci.

The fact is thus established that bacteria entering the animal body through the mouth may become implanted on an injured area within the body. The exact route which these bacteria follow is not determined.

GENERAL SUMMARY

The reproduction of streptococcic endocarditis is complete. The success of two different drugs in curing the disease in dogs, while failing to cure the disease in human beings, does not obscure the identity of the experimental disease. On the other hand, the difference in the mode of production of the experimental disease and in the mode of production of the disease in human beings is emphasized. This difference lies in the fact that bacterial implantation begins after an injury produced by trauma in the one instance and as a result of disease in the other. It seems highly important to collect a series of animals cured of streptococcic endocarditis and then to study the treatment after reinfection of the healed scars of previous infection. This will be a tedious

task. The most interesting by-product of the present study has been the demonstration of infection of traumatized valves by means of feeding streptococci to the animal. This part of the work, repeated in different years, seems adequately authenticated. It is of further interest that none of the microscopic appearances in the heart or elsewhere was such as to suggest a relation between the lesions observed in dogs and those of rheumatic fever which appear in human tissues.

LESIONS OF PERIPHERAL NERVES IN THROMBOANGIITIS OBLITERANS

A CLINICOPATHOLOGIC STUDY

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The most important manifestation of thromboangiitis obliterans is pain. To the physician its severity and persistence may present a problem that exhausts his ingenuity. To the patient it may mean not only great suffering but also incapacity for work, sleepless nights, anorexia, loss of weight, mental breakdown and finally loss of a limb. In the majority of cases in which amputation of a limb is required in thromboangiitis, it is done not because of extensive gangrene, ascending infection or septicemia but because of pain—severe, prolonged, uncontrollable, unbearable pain. Furthermore, the great majority of patients who have this disease are not "neurotic" or hypersensitive; their tolerance for pain is somewhat above the average.

Goldsmith and Brown [1] have analyzed the types of pain in thromboangiitis obliterans and have grouped them under the following headings: (1) vascular inflammatory pain, (2) pain of acute arterial occlusion, (3) intermittent claudication, (4) pretrophic pain, (5) pain of ulceration and gangrene and (6) ischemic neuritis. In the final analysis all pain in thromboangiitis obliterans must be produced by irritation, inflammation or degeneration of either sensory nerve endings or peripheral nerve trunks. However, pathologic studies of the peripheral nerves in thromboangiitis obliterans have been neglected. In spite of his classic description of the pathologic condition in the blood vessels, Buerger [2] did not mention changes in nerves except to say that when the vascular lesions become chronic, arteries, veins and nerve trunks of the legs are often bound together in a mass of fibrous tissue. Brown, Allen and Mahorner [3] mentioned briefly that fibrosis, demyelination and increase

From the Division of Medicine, the Mayo Clinic.

1. Goldsmith, G. A., and Brown, G. E.: Pain in Thrombo-Angiitis Obliterans: A Clinical Study of One Hundred Consecutive Cases, Am. J. M. Sc. **189**:819-833 (June) 1935.

2. Buerger, L.: The Circulatory Disturbances of the Extremities Including Gangrene, Vasomotor and Trophic Disorders, Philadelphia, W. B. Saunders Company, 1924.

3. Brown, G. E.; Allen, E. V., and Mahorner, H. R.: Thrombo-Angiitis Obliterans: Clinical, Physiologic and Pathologic Studies, Philadelphia, W. B. Saunders Company, 1928.

in the number of nuclei of the sheath of Schwann may occur in peripheral nerves and that inflammatory changes occasionally are present in the vasa nervorum. Diez [4] has stated that increase in the neurilemma and fibrosis of the perineurilemma of the peripheral nerve trunks are noted in thromboangiitis obliterans. He observed proliferation of the nuclei of the sheath of Schwann and resorption of myelin only in digital nerves; and because the vasa vasorum of nerve trunks appeared normal, he concluded that there was no anatomic basis for ascribing any of the pain to ischemic neuritis.

This study is based on the histopathologic observations on the peripheral nerves in 20 cases of thromboangiitis obliterans. This diagnosis was made in each case because of the presence of a clinical picture typical of the disease and because of the presence of typical histopathologic lesions of thromboangiitis in the blood vessels of the amputated specimen.

MATERIAL

The material was obtained from an amputated leg in 17 cases. Longitudinal and cross sections were made of the upper and lower portions of the anterior and posterior tibial nerves. Six of the 17 amputations had been performed above the knee, and from these legs longitudinal and cross sections were made of the popliteal nerves also. Cross sections of superficial nerves of the feet and digital nerves of the toes were taken from 7 of the legs, and cross sections of digital nerves also were examined from 2 amputated toes and 1 amputated finger. The sections of the nerves were stained with hematoxylin and eosin, Van Gieson's stain, Weigert's stain for myelin sheaths and Orlandi's stain (modified silver impregnation).

For comparison, sections were taken from popliteal nerves, upper and lower anterior and posterior tibial nerves, superficial nerves of the feet and digital nerves of the toes from 6 legs amputated because of neoplasm of the bone. The blood vessels were examined in these cases and found to be essentially normal grossly and histologically. The nerves in these legs were assumed to be normal and were used as controls.

LESIONS OF THE POPLITEAL, ANTERIOR TIBIAL AND POSTERIOR TIBIAL NERVES

Definite histopathologic changes were observed in these nerves in all but 1 case (figs. 1 to 5). They are listed under separate categories. In only a few instances was there much difference in the appearance of the anterior and that of the posterior tibial nerves at corresponding levels.

Fibrosis.—Increase of perifascicular and intrafascicular fibrous tissue was the most consistent pathologic change noted, and it occurred in all but 5 of the cases. Usually, but not always, it was more marked

4. Diez, J.: Tromboangeitis obliterante anatomía patológica, Prensa méd. argent. **21**:949-969 (May 23) 1934.

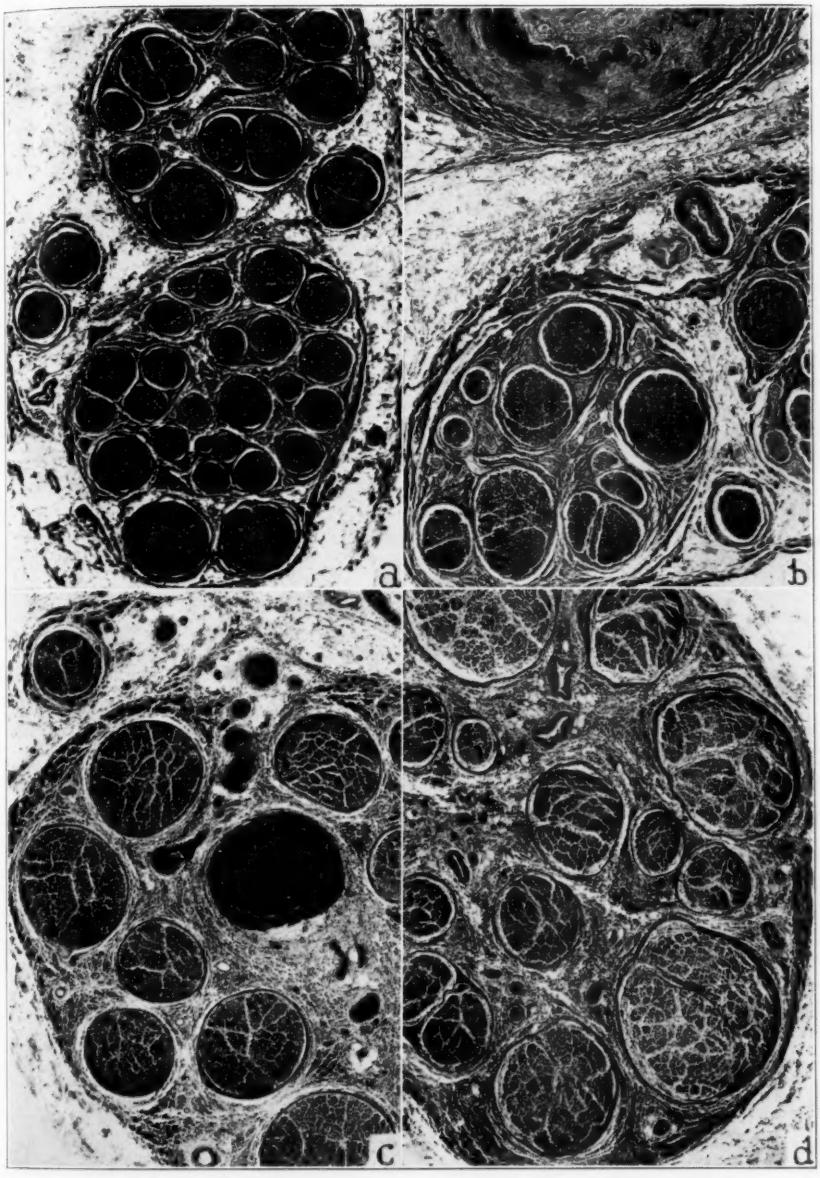

Fig. 1.—Cross sections of posterior tibial nerves; × 20. Weigert's stain for myelin sheaths was used. *a*, normal nerves. *b*, thromboangiitis obliterans, showing increased perifascicular fibrosis without definite perineural fibrosis. The completely occluded artery is also shown, and the vasa nervorum have definitely thickened walls. *c*, thromboangiitis obliterans. Perifascicular fibrosis and simple thrombosis of many of the vasa nervorum are shown. *d*, thromboangiitis obliterans. Perifascicular fibrosis, edema of the fasciculi and atrophy of the fibers are shown.

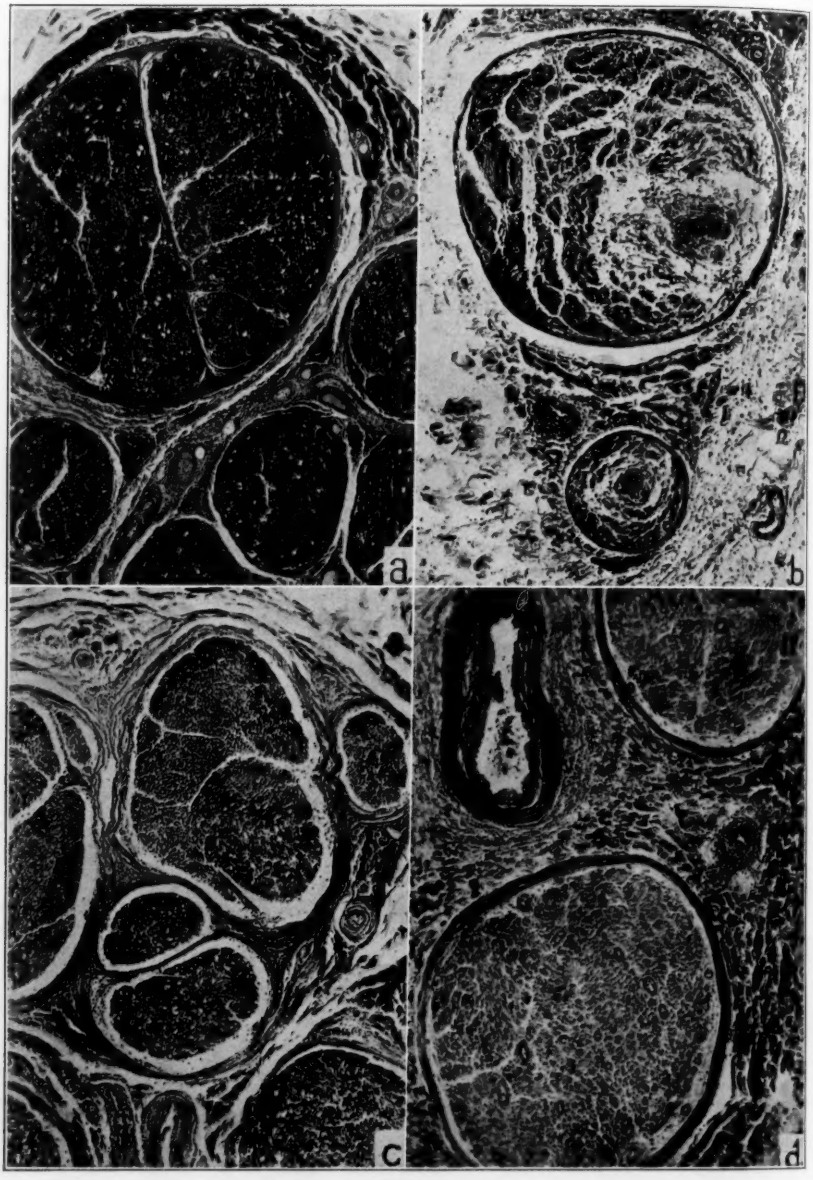

Fig. 2.—Cross sections of posterior tibial nerves; × 60; Weigert's stain for myelin sheaths. *a*, normal nerve. Note the even, dark staining of myelin. *b*, thromboangiitis obliterans. There is marked local destruction of nerve fibers inside the fasciculi, with intrafascicular edema. *c*, thromboangiitis obliterans. Note the extensive fibrosis and localized demyelination. *d*, thromboangiitis obliterans. There is almost complete demyelination of the fasciculi. Note the inflammatory reaction in the arteriole.

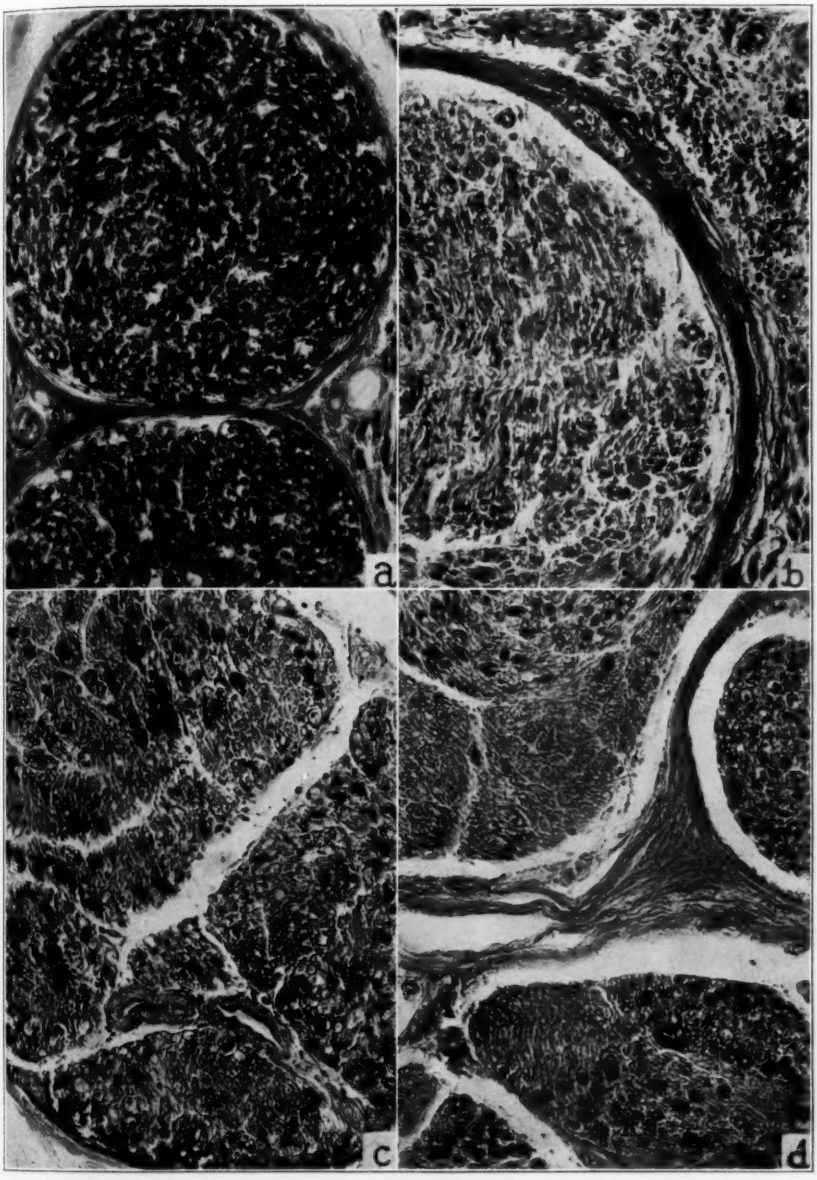

Fig. 3.—Cross sections of posterior tibial nerves; × 60; Weigert's stain for myelin sheaths. *a*, normal nerve. Note the regular arrangement of nerve fibers with deeply stained myelin sheaths. *b*, thromboangiitis obliterans. Note the edema, demyelination and destruction of nerve fibers and the numerous lymphocytes near the margin of the fasciculus in the upper left corner. *c*, thromboangiitis obliterans. Only scattered myelin sheaths remain. The vasa nervorum have definitely thick walls. *d*, thromboangiitis obliterans. Note the perifascicular fibrosis and considerable demyelination.

Fig. 4.—Longitudinal sections of posterior tibial nerves; × 160; Weigert's stain for myelin sheaths. *a*, normal nerve. Note the regular arrangement of the deeply stained myelin sheaths. *b*, thromboangiitis obliterans. Note the intrafascicular lymphocytes and marked angiitis of the small artery. *c*, thromboangiitis obliterans. Note the perifascicular and intrafascicular lymphocytes and the fragmentation of myelin. *d*, thromboangiitis obliterans. Note the fragmentation of myelin. *e*, thromboangiitis obliterans. Note the scattered lymphocytes and extensive demyelination. *f*, thromboangiitis obliterans. Note the extensive demyelination and destruction of the architecture of the nerve.

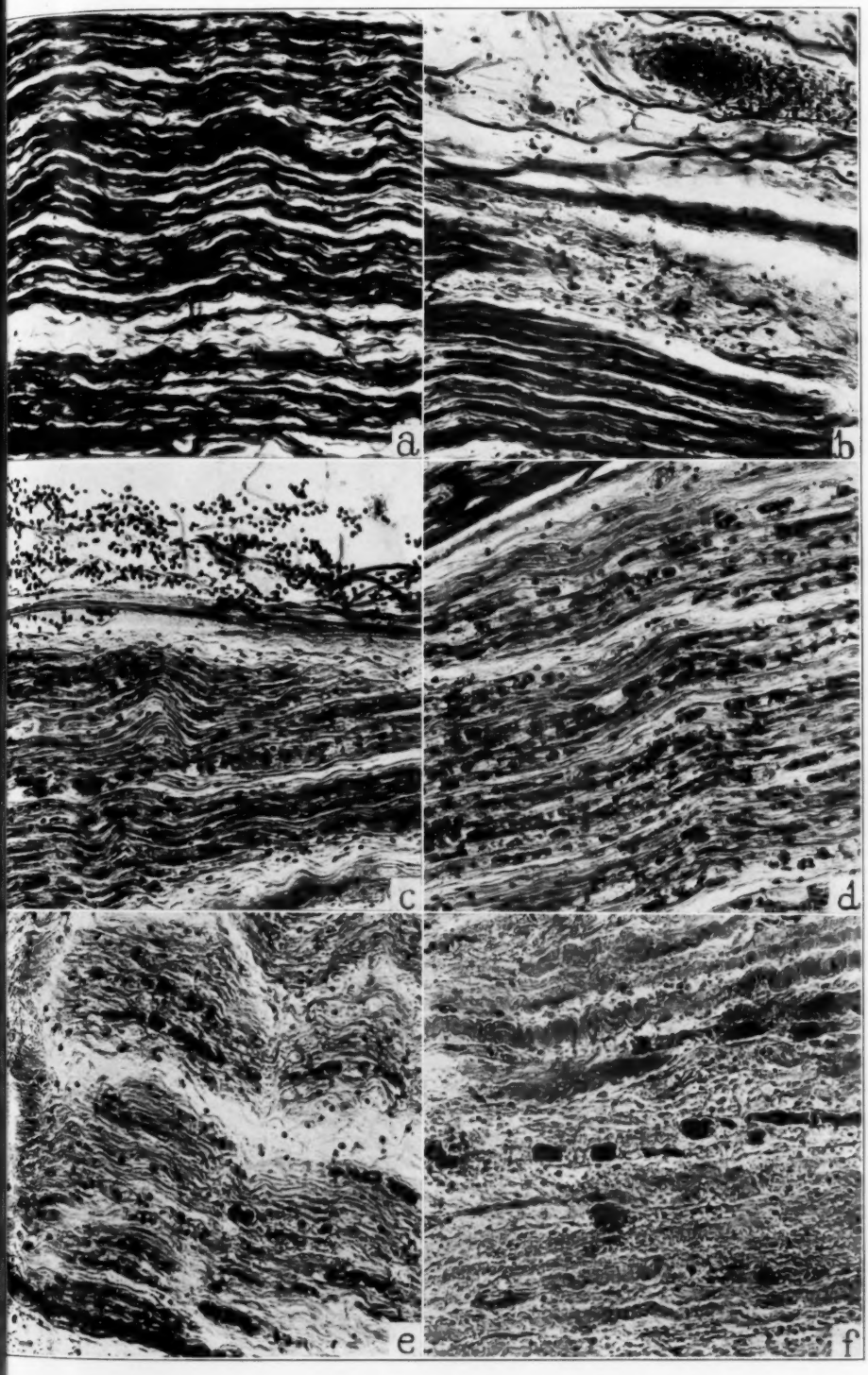

Figure 4

in distal than in proximal sections of the nerves. It is well known that fibrous tissue can exist with a meager blood supply and that it tends to be increased in all organs and tissue suffering from severe ischemia. Extensive perineural fibrosis, binding artery, vein and nerve into one dense sheath, as previously described, was not noted in any of the cases studied.

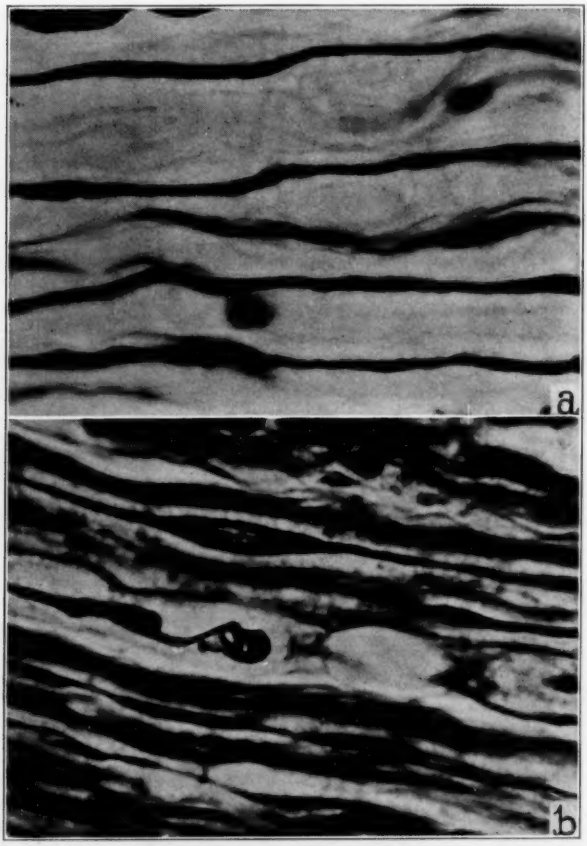

Fig. 5.—Longitudinal sections of posterior tibial nerves; × 865; Orlandi's stain (modified silver impregnation). *a*, normal nerve, showing normal axis-cylinders. *b*, thromboangiitis obliterans. Note the broken axis-cylinder with a coiled end. Also note the spindle-shaped swelling of another axis-cylinder.

Wallerian Degeneration.—It was noted that the myelin often stained irregularly in the control nerves, but complete demyelination of nerve fibers was not seen. In the nerves in 10 of the 17 cases of thrombo-

angiitis obliterans, definite areas of demyelination, with intensification of the staining reaction of the remaining myelin, were noted. This condition was usually patchy, involving certain fasciculi in a given section and sparing others. Often only part of the fibers in a fasciculus were demyelinated. In longitudinal sections, variations were noted from patchy fragmentation of myelin to complete loss of myelin and destruction of the entire architecture of the nerve, with replacement by fibrous tissue. The use of Orlandi stains was somewhat disappointing, as axis-cylinders either appeared normal or could not be identified at all, possibly owing to artefacts or changes occurring between the time of amputation and that of fixation of the tissue. In a few of the diseased nerves, however, occasional fragmented and coiled-up axis-cylinders were seen, and there were abnormal swellings of other axis-cylinders. The extent of demyelination and destruction of the nerve fibers was always greater in the distal than in the proximal segments of the nerves, but in those cases in which it was noted it was present to some extent at all levels and in all the nerves studied.

Edema and Atrophy.—Separation of the fasciculi, with thinning out of the fibrous tissue, was noted frequently. Some of this apparent change may have been due to an artefact, although it was not seen in the normal control nerves. Separation of nerve fibers themselves, with localized areas of marked atrophy and destruction, was noted in 4 of the cases in which there was extensive wallerian degeneration. This change is difficult to interpret but suggests rapid ischemic degeneration, almost infarction of the individual fasciculi.

Inflammation.—Localized collections of lymphocytes and scattered lymphocytes, around and even within the fasciculi themselves, were noted in 9 cases. These areas were patchy, and they frequently involved only one of the main trunks. Their presence did not coincide with the presence of the other changes; in fact, they were usually observed in nerve segments where there was little or no wallerian degeneration. Their presence must be interpreted as part of an inflammatory reaction but whether this is one of the first evidences of ischemic degeneration of nerves or merely of an ascending infection along the nerve sheaths from a distal gangrenous region is not clear.

Changes in the Vasa Nervorum.—Recent extensive thrombosis of the vasa nervorum was noted in 2 cases. In 6 cases small vessels, both arteries and veins, in the interfascicular and perineural regions showed the marked diffuse inflammatory reaction characteristic of thromboangiitis. Perivascular lymphocytic collections were seen only twice in the vessels inside the fasciculi. In some instances the walls of these vessels were thickened, but in the majority of instances no changes were noted. It was not possible to explain the wallerian degeneration of the nerves on the basis of ischemia due to obstruction of the vasa vasorum.

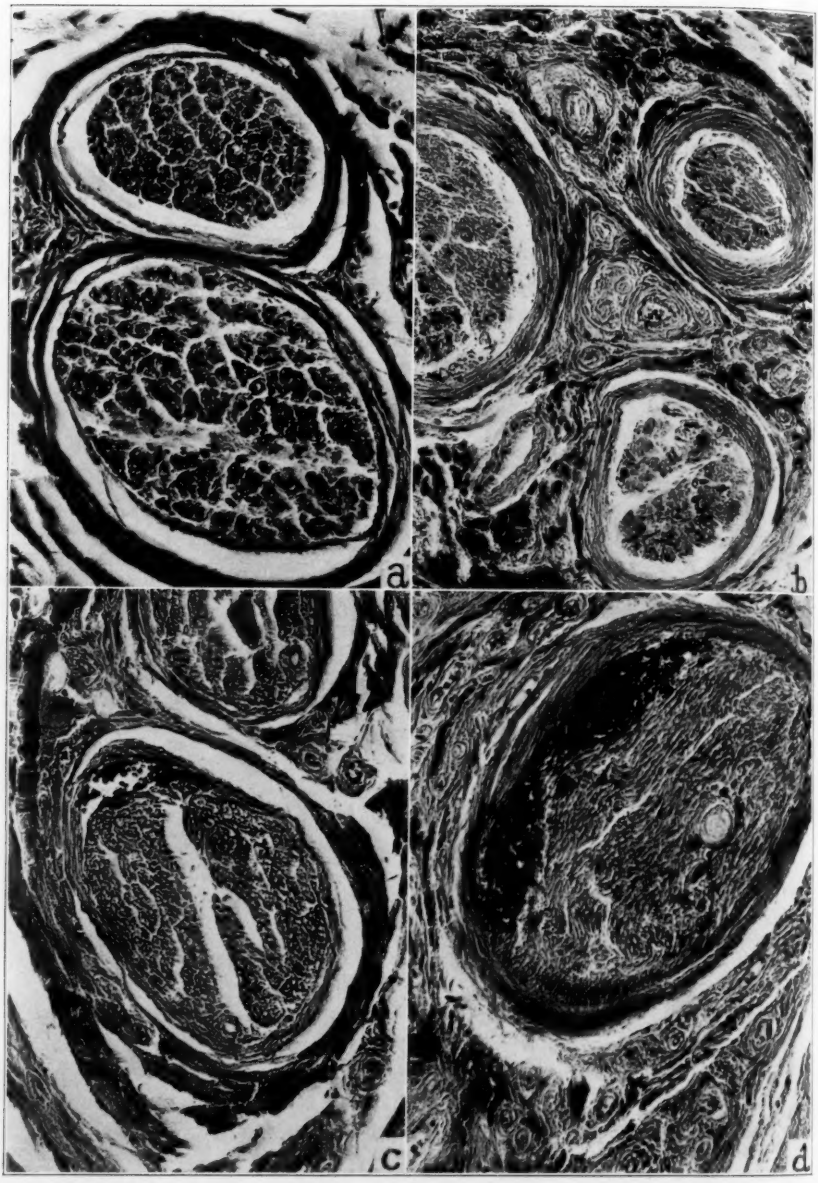

Fig. 6.—Cross sections of digital nerves; × 160; Weigert's stain for myelin sheaths. *a*, normal nerve. *b*, thromboangiitis obliterans. Note the perifascicular fibrosis and partial demyelination. *c*, thromboangiitis obliterans. There is complete demyelination. Note the perineural fibrosis and small collection of lymphocytes inside the fasciculus. *d*, thromboangiitis obliterans. There is complete demyelination. Note the perineural fibrosis and large deeply stained mass of lymphocytes inside the fasciculus.

Adequate justification for explaining all the nerve lesions on an ischemic basis exists in the extensive occlusion of the main arterial trunks which accompanied the nerves.

LESIONS OF SUBCUTANEOUS NERVES OF THE FEET AND DIGITAL NERVES NEAR GANGRENOUS REGIONS

Sections of subcutaneous nerves of the feet and of digital nerves were examined in 10 cases (table 1 and fig. 6). In 4 of these cases partial wallerian degeneration was seen in the proximal nerve trunks, and it was complete in those nerves of the foot and toes that were examined. It was also present in 2 cases in which degeneration was not seen in the proximal nerve trunks. Definitely increased perineural fibrosis was noted in all the nerves of the feet and toes. Also, it was interesting to note collections of lymphocytes inside the nerve bundles in 5 cases.

TABLE 1.—*Clinical Findings Correlated with Changes in the Nerves of the Feet and Digits*

	Clinical Data		Histopathologic Lesions in Digital Nerves and Cutaneous Nerves of Feet*				
Case	Type of Pain	Situation	Wallerian Degeneration in Proximal Nerve Trunks	Wallerian Degeneration in Local Nerves	Fibrosis	Edema	Inflammation
5	Neuritic	Leg and foot	2	4	3	0	0
6	Neuritic	Foot	3	4	3	0	1
7	Neuritic and local	Foot and fifth metatarsal	1+	4	3	2	0
8	Neuritic and local	Foot	2	4	2	1+	0
11	Local, margin of gangrene	Foot and base of toes	0	0	0	2	0
12	Local, margin of gangrene	Foot and fifth toe	0	3+	3	0	0
16	Local, margin of gangrene	Base of toe	0	2	0	3	2
18	Local, margin of gangrene	Second toe and metatarsal region	..	2	1	1	2
19	Local, margin of ulcer	Second toe	..	0	4	0	2
20	Local, margin of ulcer	Second finger	..	0	2+	1+	2

* Graded on a basis of 1 to 4.

CLINICAL ASPECTS

Goldsmith and Brown have described, under the term ischemic neuritis, a clinical manifestation which occurs in certain chronic, progressive types of thromboangiitis obliterans. In those cases there is usually partial or complete occlusion of the femoral artery. Pain is the prominent feature, and this pain occurs in large areas of the limb and is not necessarily limited to the sensory distribution of a certain nerve trunk. It is of variable intensity and occurrence but is usually severe and intractable. It is often associated with various types of paresthesia and sometimes with hypoesthesia, muscular weakness and diminution or

absence of deep reflexes. Episodes of severe vasospasm, characterized by mottled, deep cyanosis of the skin of the foot or of the entire lower portion of the leg may accompany the paroxysms of pain.

Similar pains involving the entire foot but not the leg are also occasionally described by patients. Local gangrene may be present in these cases, but the pain extends a considerable distance from the margin of the region of gangrene, tends to be paroxysmal and is usually accompanied by paresthesia.

TABLE 2.—*Clinical Findings Correlated with Changes in the Anterior and Posterior Tibial Nerves*

	Clinical Data								Histopathologic Lesions of Peripheral Nerves*					
Case	Type of Pain	Situation	Duration, Months	Vasospasm	Paresthesia	Hypoesthesia	Muscular Weakness	Diminished Reflexes	Wallerian Degeneration	Fibrosis	Edema	Lymphocytic Infiltration	Angiitis of Vasa Nervorum	Simple Thrombosis of Vasa Nervorum
1	Neuritic	Leg and foot	6	Yes	Yes	Yes	Yes	Yes	3+	3	0	0	0	0
2	Neuritic	Leg and foot	2	Yes	Yes	Yes	Yes	Yes	3	2	1	0	0	0
3	Neuritic	Leg and foot	6	Yes	Yes	0	0	0	2	3	3	0	0	3
4	Neuritic	Leg and foot	7	Yes	Yes	0	0	0	2	2	3	0	1	1
5	Neuritic	Leg and foot	2	0	0	0	0	0	2	1	1	2	2	0
6	Neuritic	Foot	2	0	0	Yes	0	0	3	0	0	0	0	0
7	Neuritic and local	Foot	3	0	0	0	0	0	1+	1	2	1	0	0
8	Neuritic and local	Foot	2	0	0	0	0	0	2	0	3	0	0	0
9	Neuritic and local	Foot	2	0	0	0	0	0	1	2	3	2	2	0
10	Neuritic and local	Foot	1	0	Yes	0	0	0	2	2	1	1	0	0
11	Local, margin of gangrene	Foot and base of toes	4	0	0	0	0	0	0	1+	2	3	2	0
12	Local, margin of gangrene	Foot and base of toes	2	0	0	0	0	0	0	1	1	0	0	0
13	Local, margin of gangrene	Foot and base of toe	1	0	0	0	0	0	0	0	0	3	0	0
14	Local, margin of ulcer	Base of toe	12	0	0	0	0	0	0	0	0	0	0	0
15	Local, margin of ulcer	Base of toe	12	0	0	0	0	0	0	2	1+	1	0	0
16	Local, margin of ulcer	Base of toe	4	0	0	0	0	0	0	0	1	3	2	0
17	Local, margin of gangrene	Base of toe	3	0	0	0	0	0	0	3	1	1	1	0

* Graded on basis of 1 to 4.

All the cases of thromboangiitis in which the nerves were studied histopathologically were also reviewed carefully from the standpoint of the type and situation of pain and the objective neurologic findings. In table 2 these data are correlated with the pathologic changes in the anterior and posterior tibial nerves.

The typical "neuritic" pain described by Goldsmith and Brown, which occurs in paroxysms and is felt in large areas of the lower portion of the leg and foot, was present in cases 1 to 5. In cases 1 to 4 there

were also extensive recurrent vasospasm and paresthesia. In cases 6 to 10 there were paroxysmal pains in the entire foot, involving regions at some distance from any local ulceration or gangrene. In the remaining cases (cases 11 to 17) the pain was localized to the region of ulceration or gangrene.

It is noteworthy that in all the cases in which the neuritic type of pain occurred, there was definite wallerian degeneration of the anterior and posterior tibial nerves and that this was not present in the cases in which there was only local pain in the regions of ulceration or gangrene. Definite correlation was not noted between the other pathologic findings—fibrosis, edema and lymphocytic infiltration, on the one hand, and the type and the situation of the pain, on the other hand. However, the lesions are definite, and they may represent stages in the process of degeneration of nerves. In cases of severe pain (cases 1 to 5) it was not possible to determine accurately the level of the nerve lesion responsible for the pain, but it is assumed that it was proximal to the level where degeneration was seen. Some wallerian degeneration was seen as far up as the level of amputation. In cases I to 5 the demyelination was increasingly more extensive in the more distal sections; therefore, it is assumed that levels of demyelination of individual fibers were scattered throughout various levels in the nerve trunks.

COMMENT

Wallerian degeneration of peripheral nerves is seen as an accompaniment of a number of lesions of nerves of different origin. It was noted by Woltman and Wilder [5] in association with diabetes. Most of their patients had definite arteriosclerotic arterial occlusions also, and the authors concluded that the basis for the lesions of the nerves was ischemia. They did not find any definite correlation between the extent of the lesions of the nerves and the clinical manifestations, such as pain, paresthesia, hypoesthesia and muscular weakness. Priestley [6] found definite wallerian degeneration as the outstanding nerve lesion in association with arteriosclerosis obliterans of the legs in nondiabetic patients. Lesions of the nerves in thromboangiitis obliterans noted in this study were considerably more extensive than those noted either by Woltman and Wilder or by Priestley, and there seemed to be a definite correlation between the presence of a neuritic type of pain and wallerian degeneration of the peripheral nerves.

5. Woltman, H. W., and Wilder, R. M.: Diabetes Mellitus: Pathologic Changes in the Spinal Cord and Peripheral Nerves, Arch. Int. Med. **44**:576-603 (Oct.) 1929.

6. Priestley, J. B.: Histopathologic Characteristics of Peripheral Nerves in Amputated Extremities of Patients with Arteriosclerosis, J. Nerv. & Ment. Dis. **75**:137-143 (Feb.) 1932.

One practical point concerns the question of surgical section of and injection of alcohol into peripheral nerve trunks, as advocated by Laskey and Silbert [7] and by Smithwick and White,[8] for the relief of intractable pain in thromboangiitis. The operation is usually done in the lower third of the leg to avoid as many motor fibers as possible. Two of the series of patients with which this paper is concerned, who had neuritic pains of the feet, had undergone section of the posterior tibial, sural, superficial peroneal and deep peroneal nerves. The pain was not relieved. This can be explained by the fact that definite wallerian degeneration was observed proximal to the level of surgical section of the nerves in both cases. It does not seem logical to expect relief of the neuritic type of pain in thromboangiitis obliterans by section of or by injection of alcohol into the peripheral nerves of the leg, as it is probable that ischemic inflammatory and degenerative lesions of nerves already have occurred at levels proximal to the site of the proposed section or injection.

The pain of ischemic neuritis is intractable to treatment, and when the lesions of the nerves are seen, this is not difficult to understand. Occasionally, after a period of weeks or months, it disappears spontaneously, possibly owing to ultimate cessation of degeneration. It is not known how completely regeneration may occur.

SUMMARY

In a histopathologic study of the peripheral nerves in a series of 20 cases of thromboangiitis obliterans, various combinations of wallerian degeneration, fibrosis, edema, atrophy, lymphocytic infiltration, inflammation and thrombosis of the vasa vasorum were noted in all but 1 case. A definite correlation was found between the presence of wallerian degeneration and the clinical syndrome of ischemic neuritis.

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7. Laskey, N. F., and Silbert, S.: Thrombo-Angiitis Obliterans: Relief of Pain by Peripheral Nerve Section, Ann. Surg. **98**:55-69 (July) 1933.

8. Smithwick, R. H., and White, J. C.: Peripheral Nerve Block in Obliterative Vascular Disease of the Lower Extremity: Further Experience with Alcohol Injection or Crushing of the Sensory Nerves of the Lower Leg, Surg., Gynec. & Obst. **60**:1106-1114 (June) 1935.

BOECK'S SARCOID

REPORT OF A CASE, WITH CLINICAL DIAGNOSIS CONFIRMED AT AUTOPSY

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AND
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The scarcity of autopsy material in connection with Boeck's sarcoid is explained by the fact that sarcoid runs a slow course, most often showing a preference for cutaneous manifestations. Nickerson,[1] in 1937, reported 6 cases in which autopsies were performed, describing for the first time lesions in the myocardium, endocardium, pancreas, testis and vertebral and femoral marrow, also differentiating the microscopic picture of sarcoid from that of tuberculosis.

Hunter [2] recently reported a case of sarcoid, with a review of the historical literature. His patient presented extensive changes in the lungs which completely cleared up under a strict hygienic regimen.

Longcope and Pierson,[3] in reporting 8 cases, in 7 of which there were pulmonary changes, included a full bibliography. They stressed the importance of recognizing that although the disease is more often described as a disease of the skin, the generalized nature of the process is to be borne in mind.

Since the publication, in 1915, of the article by Kuznitzky and Bittorf [4] demonstrating the pulmonary changes by means of roentgenograms for the first time, there have been numerous communications establishing the disease as a generalized condition. The disease may be recognized not only by the cutaneous lesions but also by manifestations elsewhere, such as pulmonary, osseous or ocular changes, which may give rise to the presenting symptoms.

From the New England Deaconess Hospital.

1. Nickerson, D. A.: Boeck's Sarcoid: Report of Six Cases in Which Autopsies Were Made, Arch. Path. **24**:19-29 (July) 1937.

2. Hunter, F. T.: Hutchinson-Boeck's Disease (Generalized "Sarcoidosis"): Historical Note and Report of Case with Apparent Cure, New England J. Med. **214**:346-352, 1936.

3. Longcope, W. T., and Pierson, J. W.: Boeck's Sarcoid (Sarcoidosis), Bull. Johns Hopkins Hosp. **60**:223-296, 1937.

4. Kuznitzky, E., and Bittorf, A.: Boecksches Sarkoid mit Beteiligung innerer Organe, München. med. Wchnschr. **62**:1349-1353, 1915.

Doub and Menagh,[5] in 1929, reported 2 cases in which the bones of the hands and feet showed cystic changes. Mylius and Schürmann,[6] in 1929, reported 2 cases of sarcoid in which the patients were followed clinically and later came to autopsy. In case 1 the first manifestation was iritis. Later in the course of the disease the osseous and pulmonary changes were demonstrated by roentgen examination. At autopsy, tuberculosis, in addition to sarcoid, was evident. In case 2, autopsy was limited to the chest, revealing huge glands which showed only sarcoid. There were rare foci of caseation in the lungs. From their experience the authors said they felt that sarcoid is a special type of tuberculosis. Hantschmann,[7] in 1930, reported 6 cases of sarcoid with pulmonary changes. Kirklin and Morton,[8] in 1931, presented several cases in which osseous and pulmonary changes were well illustrated. Kissmeyer's [9] monograph, published in 1932, gave a complete presentation with numerous illustrations.

REPORT OF CASE

History.—Our patient was 1 of 6 children, 3 of whom were still alive and well; 1 had died of cardiac disease and 1 had died of Hodgkin's disease (sarcoid?). Our patient died at the age of 51. Prior to this illness he had been well except for "neuritis" at the age of 34 and appendicitis at the age of 43. He married at the age of 40, and there were no children.

The onset of the illness, in May 1934, was characterized by pain, swelling and stiffness in the hands and feet, and fever of three weeks' duration, the symptoms gradually becoming worse. He showed rather severe generalized periarticular involvement, which was partially ameliorated by the use of salicylates and aminopyrine. During this illness there were progressive anemia, loss of weight, weakness and cough.

Physical Examination.—Examination revealed fusiform swelling of the fingers and wrists and edema of the ankles. The liver was palpable 3 fingerbreadths below the costal margin. There was generalized peripheral glandular enlargement, especially in the axillary and inguinal regions.

Laboratory Study.—Extensive laboratory studies were made. The red blood cell count was 4,000,000, the hemoglobin value was 70 per cent, the white blood cell count was 20,000, the urine was normal, the Wassermann and Kahn tests

5. Doub, H. P., and Menagh, F. R.: Bone Lesions in Sarcoid: A Roentgen and Clinical Study, Am. J. Roentgenol. **21**:149-155, 1929.

6. Mylius, K., and Schürmann, P.: Universelle sklerosierende tuberkulöse grosszellige Hyperplasie, eine besondere Form atypischer Tuberkulose, Beitr. z. Klin. d. Tuberk. **73**:166-209, 1929.

7. Hantschmann, L.: Ueber torpide Formen disseminierter Tuberkulose, Beitr. z. Klin. d. Tuberk. **73**:688-709, 1930.

8. Kirklin, B. R., and Morton, S. A.: Roentgenologic Changes in Sarcoid and Related Lesions, Radiology **16**:328-333, 1931.

9. Kissmeyer, A.: La maladie de Boeck: Sarcoïdes cutanées benignes multiples, Copenhagen, Levin & Munksgaard, 1932.

gave a negative reaction, the gonococcus complement fixation test gave a doubtful reaction, the sedimentation time was twenty to thirty minutes, the cultures of blood were sterile and chemical study of the blood gave normal values.

Roentgen examination of the chest (fig. 1) revealed dense symmetric bilateral glandular enlargement with fine lacelike infiltration extending out from roots of both lungs. The appearance of the chest was that of lymphoblastoma. Studies of the gastrointestinal tract revealed no unusual findings. Retrograde pyelography also showed a normal condition. Examination of the skeleton revealed hypertrophic changes about the bodies of the twelfth dorsal and the first and second lumbar vertebrae and both hips. Intravenous injection of dye showed a normal gallbladder. Roentgenograms of the hands and wrists were normal. There was narrowing

Fig. 1.—Roentgenogram of the chest taken at the onset of the illness, showing the huge bilateral symmetrically enlarged hilar nodes, with cordlike infiltration extending out into the parenchyma of the lung.

of the joint space of the right knee. The sinuses were normal. In view of the changes in the lungs suggesting lymphoblastoma, roentgen therapy was given over this area. Since the glands did not disappear, it was thought that the changes were not those of lymphoblastoma. In view of the infectious arthritis, typhoid vaccine was given, with some improvement.

Course.—The patient was discharged improved from the hospital. The diagnosis was infectious polyarthritis, hypertrophic osteoarthritis and bilateral adenitis of the roots of the lungs.

Shortly after discharge he came under our observation, and in addition to the aforementioned data, it was learned that the gonococcus complement fixation test gave a positive reaction. The patient repeatedly denied the possibility of

infection. Subsequent tests gave positive results. He had been free from cough for one week, and the anemia had subsided. The striking features of the physical examination were edema of the ankles and fusiform swelling of the phalangeal joints. At the time there was no enlargement of the peripheral nodes. With a high protein diet the edema subsided and the joints became flexible, and in October 1934 it was thought he would soon regain his health.

On October 23 the patient had renal colic due to a small calculus. In November there was return of the articular symptoms, with subsidence in January. At this time there were symptoms of a duodenal ulcer, and the diagnosis was confirmed roentgenographically. Despite hospitalization and dietary treatment, the ulcer failed to respond to medical management, and in May 1935 posterior gastroenterostomy was necessary. This operation was completely successful, and there

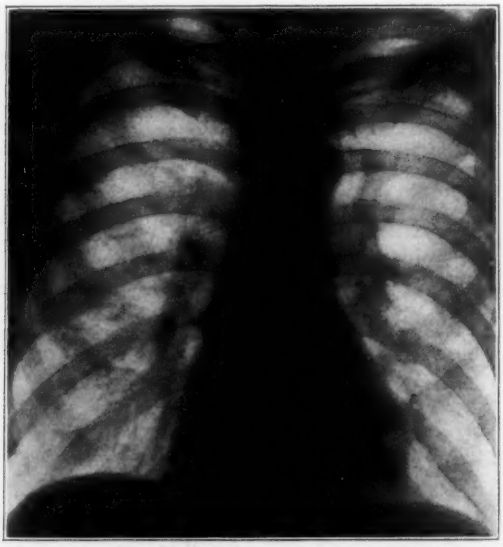

Fig. 2.—Roentgenogram of the chest taken ten months later than the one shown in figure 1. The appearance is essentially normal.

were no further symptoms referable to the duodenal ulcer. During this time the patient was free from symptoms referable to the joints. A roentgenogram of the chest was entirely normal, no enlargement of the hilar nodes being visible (fig. 2).

Four weeks after operation (June 1935) there was a recurrence of the arthritic symptoms. He was again given a high protein diet. He was not seen for four months, after which he reported that the arthritis had lessened while he was living at the seashore in July but was worse in August. He showed recurrence of the fusiform swelling of the phalangeal joints, with marked limitation and similar involvement of the right elbow. He went to Florida for the winter, remaining until June 1936. He complained of swelling of both forearms and of inability to close his hands. He could walk only with pain. Laboratory studies at this time revealed no anemia. The calcium content of the

serum was 10.1 mg. per hundred cubic centimeters. The protein content of the plasma was 6.6 mg. per hundred cubic centimeters. The gonococcus complement fixation test gave a positive reaction. The tuberculin test gave a negative reaction. It was thought that the edema might be due to nutritional factors, despite the normal plasma protein value. He was given a high protein diet, without improvement.

In July there was definite secondary anemia, and the articular symptoms had increased. Shortly after this he had a severe cough and fever and was confined to bed.

Roentgen examination of the chest (fig. 3) revealed the same picture as in 1934, and a diagnosis of sarcoid was made. On roentgen examination the hands

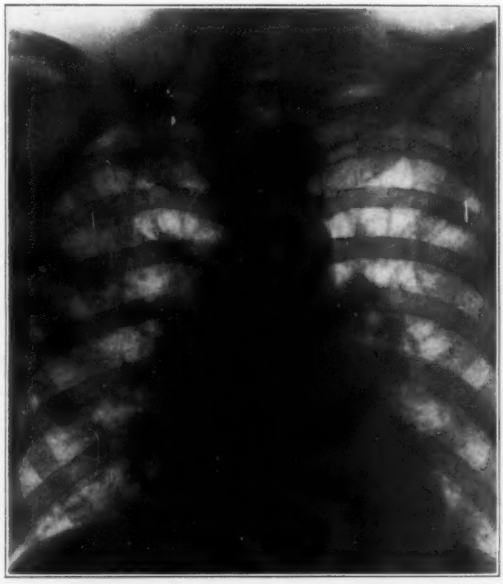

Fig. 3.—Roentgenogram of the chest taken two years and two months after the onset of the illness. The picture is essentially similar to that in figure 1. The discrete nodes are readily visible.

and feet appeared normal. At this time he showed over the forearms faint dry, scaly erythematous patches which were not characteristic of sarcoid and which suggested a tentative diagnosis of xeroderma. The edema was less. The cough was his chief symptom.

The patient's condition changed little until one week before death, when edema of the larynx developed, with a change in voice. On Oct. 22, 1936, two and a half years after the onset of his symptoms, the patient collapsed, with air hunger and profuse sweating, and died in thirty minutes.

Gross Postmortem Examination.—Autopsy was performed ten hours post mortem.

General: The body was that of a slightly undernourished but normally developed man, about 140 pounds (63.5 Kg.) in weight and 5 feet and 8 inches (173 cm.) in height. The skin over the arms, chest and back showed light brown pigmentation and scaling, somewhat more prominent over the chest anteriorly. The scrotum was edematous. The extremities were normally developed but showed some irregular swelling of the knee and ankle joints. The greater portion of swelling appeared to be edema of soft tissues, most pronounced about the ankles and feet. The phalangeal joints of the hands were irregularly prominent. Exploration of the right knee disclosed smooth articular surfaces and normal-appearing synovial fluid.

Lungs: The right pleural cavity contained 750 cc. and the left 500 cc. of serous slightly yellow fluid. The lungs were relatively voluminous. There were no adhesions. The lungs weighed 800 Gm. each. The pleurae were smooth and grayish blue and not infrequently finely granular, owing to a minute grayish white hyaline deposition. In addition, over both apexes, especially posteriorly, there were large plaques, the largest being 3 cm. long and 8 mm. thick, of slightly elevated grayish white hyalin. This was not associated with scarring of the underlying lung. Two subpleural calcified nodules, about 5 mm. in diameter, were at the posterior aspect of the lower lobe of the left lung 3 cm. from the diaphragmatic margin. Similar nodules occurred posteriorly in the lower lobe and in the middle lobe of the right lung, but these calcified nodules on section showed also anthracosis. On section all the lobes were moderately subcrepitant, relatively firm and pinkish gray, with small poorly defined firmer foci of gray. A moderate amount of slightly blood-tinged fluid could be expressed from the cut surface. Occasionally throughout the lung, nodules, 3 mm. or less in diameter, were encountered which were firm and black. The bronchi were filled with yellowish brown mucus, and the mucosa was swollen. The trachea and larynx, including the epiglottis, exhibited edema. The pulmonary arteries and veins were normal.

Heart: The pericardial cavity contained 75 cc. of clear slightly yellow serous fluid. The pericardium was thin, smooth and glistening throughout. The heart weighed 375 Gm. and was normal except for moderate coronary sclerosis.

Spleen: The spleen weighed 175 Gm. The capsule was slightly wrinkled, smooth and gray. The cut surface was moderately soft and grayish red. The trabeculae were indistinct. The follicles were small and generally poorly defined.

Gastrointestinal Tract: The gastrointestinal tract was normal except for the old posterior gastrojejunostomy, with jejunal ulcer.

Liver: The liver weighed 1,500 Gm. The capsule was thin, smooth and reddish brown. The cut surface was flat, normally lobulated and reddish brown. The central zones were indistinct.

Lymph Nodes: The bronchopulmonary lymph nodes were enlarged bilaterally and symmetrically, the largest being 2.5 cm. in diameter. They were discrete and soft and on section presented a bulging moist pink-gray surface which was homogeneous except for an occasional small focus of soft gray tissue. Inferior to the bifurcation of the trachea the lymph nodes were similarly enlarged, the largest being 3.5 cm. in length. The nodes superior to the bifurcation and the peritracheal nodes presented a similar appearance and were up to 1.5 cm. in length (fig. 4). No nodes were palpated in the neck or axillae. Abdominal periaortic and iliac lymph nodes were similarly enlarged, the largest being 2 cm. in greatest diameter. One node in the celiac region showed a focus of gray fibrous tissue 7 mm. in diameter. The inguinal nodes were slightly enlarged and appeared similar to those elsewhere. The portal, gastric and mesenteric lymph

nodes showed no enlargement. Bacteriologic examination of a bronchopulmonary node revealed no tubercle bacilli, and guinea pig inoculation gave negative results.

Bones: The lumbar vertebrae and ribs showed no abnormality. The marrow of the lumbar vertebrae was normal.

Microscopic Postmortem Examination.—The microscopic examination showed the following:

Heart: Section of the left ventricle showed muscle fibers of average width or slightly attenuated, with no enlargement of the nuclei. Throughout the myo-

Fig. 4.—A retouched photograph of the gross specimen. The posterior aspect of the lungs shows the enlarged nodes, below the bifurcation, measuring 4.5 cm. in greatest diameter.

cardium were numerous, usually small and frequently perivascular areas of connective tissue which often appeared edematous and mildly infiltrated with lymphocytes and a few eosinophils. There was slight extension of epicardial fat into the myocardium. Occasionally within the connective tissue were small foci of irregularly oriented epithelioid-like cells, without giant cells and caseation but

with necrosis of collagen and a few lymphocytes. In the plane of this section the foci were not related to the blood vessels. The capillaries adjacent to the foci showed slight reduplication of the endothelium. The epicardium was thickened, and throughout the epicardial fat was a scattering of lymphocytes, with a few leukocytes, chiefly eosinophilic, which were more numerous near a large coronary artery. There was considerable intimal and medial thickening of the two large coronary arteries, accompanied by hyalinization and lipoid deposition. Arteries within the myocardium were generally not appreciably thickened.

Lung: Sections from the upper and lower lobes of the lungs exhibited similar histologic pictures. Scattered throughout, frequently in groups and somewhat more numerous in the vicinity of the small blood vessels and the bronchi, were numerous miliary lesions, which tended to remain in the alveolar wall. The lesions were typified by small collections of epithelioid cells which centrally were irregularly arranged and peripherally showed a disposition to concentric arrangement. Infrequently there were giant cells with three to fifteen nuclei simulating foreign body giant cells. Occasionally cells within the lesions were pigmented. There was no caseation, and the lesions tended to remain discrete, frequently being surrounded by collagenous connective tissue. Scattered throughout the connective tissue were a variable but generally small number of small mononuclear cells, chiefly lymphocytes. Intervening lung showed empty alveoli except for a scattering of desquamated cells and a few red blood cells. In some foci the alveoli were partly collapsed, and a few were hyperdilated. Some portions of the pleura showed no involvement or only an occasional lesion immediately beneath the pleura. The pleura of the upper lobe of the right lung was greatly thickened by fibrous and hyalinized connective tissue, throughout which were collections of small mononuclear cells, chiefly lymphocytes; only one group of cells that were suggestive but not typical of nodules was seen elsewhere. The section of the upper lobe of the left lung included a small pulmonary artery in which was hemispherical elevation of the intima, comprised of mononuclear cells, some of which were similar to those of the lesions previously described but in atypical arrangement. There were also a few lymphocytes. The remaining arteries and veins appeared normal.

The bronchi were normal although there were many peribronchial lesions. A section of the lower lobe of the left lung showed healed lesions resembling a primary tubercle, with central caseation and calcification and a peripheral zone of dense hyalinized connective tissue. There was considerable fibrosis of the adjacent lung. Another section of the lower lobe of the left lung showed similar scarring and a portion of a calcified focus surrounded by dense connective tissue. A section of the lower lobe of the right lung showed a subpleural nodule surrounded by dense hyalinized connective tissue and centrally comprised of acidophilic shadowy structural outlines, scattered throughout which was a small amount of black pigment. There was mild fibrosis in the immediate vicinity, with mild lymphocytic infiltration and occasional lesions not typical of tubercles but similar to and less poorly defined than the lesions previously noted.

The trachea showed numerous lesions both immediately beneath the mucosa and among the mucous glands, where there were considerable distortion, fibrosis, round cell infiltration and degeneration. The lesions here exhibited less tendency to peripheral concentric arrangement of cells, and the cells were more frequently irregularly than radially arranged. The underlying stroma was edematous, and the vessels were slightly hyperemic.

Spleen: The capsule was normal. Scattered throughout the spleen were numerous lesions comprised of epithelioid cells without caseation. The cells

were irregularly disposed, and there was no constant disposition to concentric peripheral arrangement. The periphery was generally surrounded by a few lymphocytes and was poorly defined from the adjacent pulp. Generally the stroma and the red pulp were moderately dense. The sinuses for the most part were empty, although occasionally they contained red blood cells. The malpighian

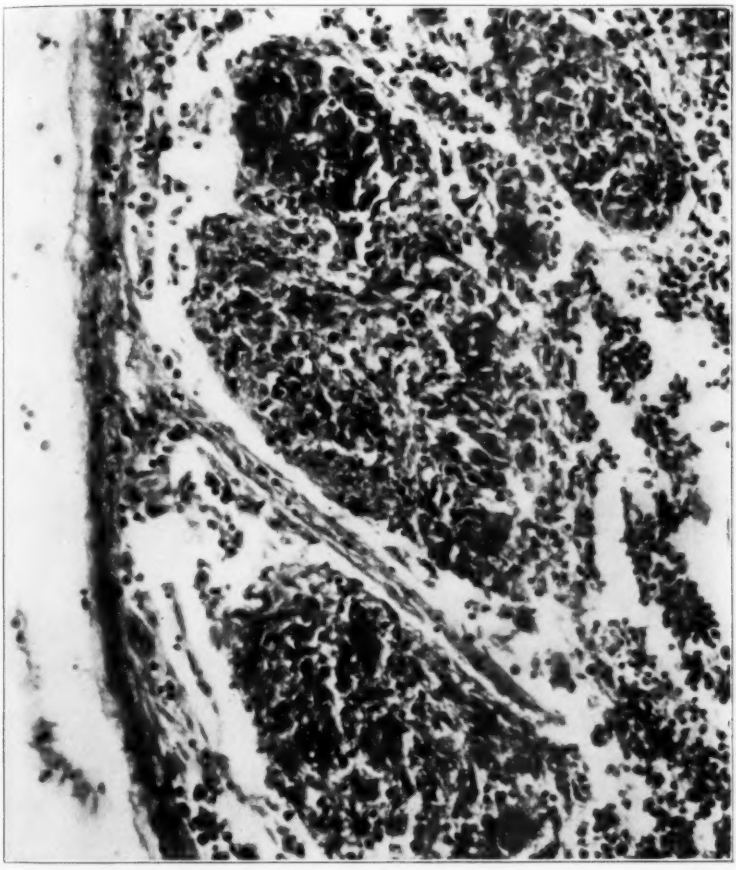

Fig. 5.—Photomicrograph of a section of the margin of a hilar node, showing sarcoid nodules and edema; × 300.

bodies exhibited slight activity and were moderately numerous. The trabeculae were small and appeared normal.

Liver: There was frequently an increase of connective tissue in the portal zones. Scattered irregularly throughout the liver and confined to no particular zone were numerous lesions similar to those just described. Some showed con-

centric and others irregularly radial disposition of the peripheral cells, and there was frequently mild lymphocytic but rarely leukocytic infiltration at the periphery. Giant cells similar to those previously described were occasionally encountered within the lesion. Frequently there was a small amount of concentric collagen in the peripheral portion.

Fig. 6.—Photomicrograph of a section through the center of a hilar node, showing edema and dilated sinuses; × 48.

Kidney: Three sections from the kidneys showed a similar picture. Infrequently within the cortex and medulla were lesions similar to those already described.

Lymph Node: Sections of portal, abdominal, periaortic, peritracheal and bronchopulmonary lymph nodes and lymph nodes inferior to the bifurcation of the

trachea included areas of essentially similar change. There were numerous lesions similar to those described in the lungs although showing more variation. The lesions varied from small usually rather distinct collections of epithelioid-like cells, centrally irregularly arranged and peripherally either concentrically or irregularly radially disposed, to larger poorly defined lesions, with a few identifiable cells distributed throughout an acidophilic hyaline matrix that was usually rich in collagen (fig. 5). Usually there was some peripheral collagen, and not infrequently there was some acidophilic intercellular hyalin centrally. There were giant cells of foreign body type occasionally within the lesions. No caseation but necrosis of collagen was noted. Where the lesions were numerous and widespread, much of the architecture of the node was obliterated by fibrosis. This was a prominent feature of the bronchopulmonary nodes. Otherwise the nodes exhibited marked edema and slight hyperplasia (fig. 6). The sinuses within the nodes and the external lymphatic vessels were widely dilated and frequently contained fibrin. Section of a left inguinal node showed marked edema and dilatation of the sinuses but no definite lesions.

Skin: Section of the skin from the chest showed a few small round cells about slightly ectatic vessels in the superficial corium. Immediately beneath the corium in the fat and not apparently in relation to the vessels were a few lesions similar to those just described. The basal cell layer of skin contained an increased amount of pigment. A scattering of cells in the papillary layer contained golden brown rather refractile pigment. The arteries were normal.

Aorta: The intima and the inner portion of the media exhibited slight degeneration.

Thyroid Gland: Several small lesions similar to those just described were irregularly scattered throughout the gland; otherwise it was normal. The parathyroid glands were normal.

Bone: Sections of a lumbar vertebra and a rib showed slightly hyperplastic marrow.

Final Diagnosis.—The final diagnosis was sarcoid, with involvement of the bronchopulmonary, tracheal, paravertebral, iliac and inguinal lymph nodes, lungs, trachea, heart, spleen, liver, thyroid gland, kidneys and skin; edema of the larynx and trachea; bilateral hydrothorax; a healed primary tuberculous complex (calcified), and duodenal and jejunal ulcers (with old posterior gastroenterostomy).

COMMENT

In this case, greatly enlarged bilateral hilar lymph nodes were at first presented; these cleared up completely and later recurred. Moreover, the autopsy revealed the mechanism involved, an intense and extensive edema of the nodes, with the presence of sarcoid lesions (fig. 6). The roentgenograms and the photographs of the gross specimen (figs. 1 to 4) plainly showed the large size and discrete character of the nodes, one measuring 4.5 cm. in diameter, largely because of edema.

There was no evidence of active tuberculosis, in spite of vigorous search and animal inoculation. A tuberculin test two years before death gave a negative reaction. A healed primary pulmonary lesion, probably tuberculous, was present.

For the first time lesions in the kidney and thyroid gland are reported.

Extensive and characteristic changes in the lungs, with surprisingly few symptoms, are usually present. The persistent cough in this case, an unusual feature, was probably due to the tracheal lesions, which were demonstrated at autopsy. The striking changes in the lungs, thought at first to be due to Hodgkin's disease, gave an immediate response to irradiation that was discouraging, yet in less than one year the roentgenographic appearance of the chest was absolutely normal (fig. 2). After another year the patient became definitely worse, with progressive arthritis. At this time there was a recurrence of the pulmonary changes, and the true nature of the condition was determined.

SUMMARY

A case of Boeck's sarcoid is presented in which the patient was followed for two years, the diagnosis was made ante mortem and autopsy confirmed it. Lesions were present in the lung, skin, liver, spleen, myocardium, lymph nodes, trachea, thyroid gland and kidney. Edema of the lymph nodes may be an important factor in their enlargement. This case clarifies the mechanism involved in huge though variable lymph nodes.

It is again emphasized that sarcoid may be generalized and may present changes sufficient to make a tentative diagnosis possible not only on the basis of the cutaneous manifestation but also from changes in the lungs as well as in the bones.

ORAL RAGWEED POLLEN THERAPY

CLINICAL RESULTS OF EXPERIMENTS ON GASTROINTESTINAL ABSORPTION

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The evident disadvantages of hypodermic administration—discomfort, expense and liability to reactions—have resulted in repeated attempts to substitute oral administration in immunotherapy. Much has been said concerning the oral use of typhoid vaccine, cold vaccines and extracts of poison ivy. Although there is no evidence of great effectiveness of this method in the prevention of typhoid, the common cold and rhus dermatitis, the simplicity of the procedure has aroused interest among members of the medical profession and the public. It is but natural, therefore, that for a disease as common as hay fever such treatment should be suggested and tried.

In 1922 Touart [1] reported the results of treatment of 6 patients with hay fever by daily ingestion of a tablet containing 0.1 mg. of pollen protein coated with phenyl salicylate. The patient with allergy to grass pollen obtained relief, but only 1 of those with allergy to ragweed pollen was relieved. Black,[2] experimenting on himself, found that ragweed antigen could be demonstrated in the blood and urine after the ingestion of fairly large amounts of pollen extract. The method he used to demonstrate ragweed antigen in the serum was to test himself intradermally with his own serum and also to test with the same serum the skin of a passively sensitized nonallergic person. Later, Black [3] employed oral therapy for several patients with grass or ragweed sensitivity and compared the results with those for patients treated by the hypodermic method. Complete failures occurred in 20 per cent of the cases with oral therapy as against 6 per cent with the injection method.

From the Department of Medicine, Northwestern University Medical School.

1. Touart, M. D.: Hay Fever: Desensitization by Ingestion of Pollen Proteins, New York M. J. **116**:199 (Aug. 16) 1922.

2. Black, J. H.: The Oral Administration of Pollen, J. Lab. & Clin. Med. **12**:1156 (Sept.) 1927.

3. Black, J. H.: The Oral Administration of Pollen: A Clinical Report, J. Lab. & Clin. Med. **13**:709 (May) 1928.

In 22.2 per cent of the cases of pollen asthma, complete failure resulted with oral therapy, while with the hypodermic method no absolute failures were noted.

In his treatise on hay fever Thommen [4] cited a satisfactory result in a case of hay fever in which pollen was given orally. Such attempts in other cases gave variable results. Thommen was not favorably impressed with this method because of the large amounts of extract required and the variability of enteral absorption. Urbach [5] has published a number of communications in which he has claimed to have obtained satisfactory results in hay fever by the use of peptones of the specific pollen administered orally. He has also reported similar results with the use of peptones made from the entire pollinating flower.

In 1933 and 1934 Gatterdam [6] reported on the use of oral pollen therapy. In 75 to 85 per cent of a series of 85 patients thus treated marked relief was obtained. The pollen was given twice daily, with additional doses during attacks. The usual amount was from 3 to 15 drops of a 3 per cent extract. Occasionally mild urticaria or hay fever resulted from overdosage. The pollens used were those of cottonwood, ash, Bermuda grass, rabbit bush and false ragweeds.

The fact that a number of hay fever sufferers had claimed that they obtained relief by eating honey produced in their vicinity led McGrew [7] to suspect that the pollen in the honey was responsible for the result. He therefore tried oral pollen therapy. A 1 per cent extract of pollen was employed coseasonally, 1 to 10 drops being given three times daily or for the immediate relief of an individual attack. Overdosage caused aggravations of symptoms. Of 33 patients thus treated, 29 were improved. Those with multiple allergy or with asthma did not do so well.

The most favorable report was that of Stier and Hollister,[8] who obtained satisfactory results in 78 per cent of a large series of cases in an experience extending over three years. Their best results were

4. Thommen, A. A.: Asthma and Hay Fever in Theory and Practice, Springfield, Ill., Charles C. Thomas, Publisher, 1931, p. 764.

5. Urbach, E.: Desensibilisierung pollenallergischer Individuen auf oralem Wege mittels artspezifischer Pollenpeptone, Klin. Wchnschr. **10**:534 (March 21) 1931; Die Behandlung des Heufiebers mittels artspezifischer Gräserblüten-Propeptane bzw. Pollen-Mikromahlzeiten, ibid. **12**:1797 (Nov. 18) 1933; Pathogenese und Therapie des Heufiebers, Wien. klin. Wchnschr. **47**:1073 (Aug. 31) 1934; Die biologische Behandlung des Heufiebers, München. med. Wchnschr. **84**:488 (March 26) 1937.

6. Gatterdam, E. A.: Oral Administration of Pollen Extracts, Southwestern Med. **17**:199 (June) 1933; Hay Fever in Central Arizona and Its Treatment with Oral Extracts, ibid. **18**:130 (April) 1934.

7. McGrew, G. D.: Time and Money Saved in the Treatment of Hay Fever, Mil. Surgeon **80**:371 (May) 1937.

8. Stier, R. F. E., and Hollister, G.: Desensitization by Oral Administration of Pollen Extracts, Northwest Med. **36**:166 (May) 1937.

obtained with the coseasonal method of administration. The pollens used consisted of a large variety but did not include ragweed pollen. The doses consisted of 3 drops of a 1 : 100,000 dilution of pollen to a maximum dose of 21 drops of a 1 : 100 dilution. Most patients required a quantity of the 1 : 100 solution daily. In a few instances mild systemic symptoms were observed.

Bernstein and Kirsner,[9] administering a 5 grain (0.3 Gm.) capsule of ragweed pollen orally, were unable to demonstrate enteral absorption sufficient to cause a reaction in the passively sensitized skin of 4 nonallergic persons. No mention was made of any attempt to compare by the same technic the presence of the ragweed antigen in the circulation after it had been administered hypodermically. These authors also corroborated previously recorded observations that digestion of pollen by gastric juice caused only a moderate diminution of the cutaneous reactivity of ragweed pollen.

Because almost all the successful results concern districts either entirely free from ragweed pollen or having comparatively little of it and because as a rule protection to pollen allergy other than that of ragweed is more easily attained, it was thought best to make a study of oral therapy of patients with ragweed sensitivity in a community representative of the Middle West.

EXPERIMENTS ON GASTROINTESTINAL ABSORPTION OF POLLEN

It was deemed advisable by us to make studies on gastrointestinal absorption of pollen before we ventured into the problem of oral therapy. This we considered essential in order to have at least a rough estimate of the doses to be used clinically.

We employed for this purpose a method previously used by us[10] in demonstrating quantitatively the absorption rate and tide of injected antigens. In the experiments reported earlier we sensitized a number of sites on several persons with serial dilutions of serum containing ragweed reagin. Within a few minutes after the injection of a ragweed extract, reactions began to appear in the sensitized areas. The appearance time of the first reaction, the time at which the last reaction took place and the highest dilution of reagin at the site of which a reaction was obtained gave an indication of the rate and degree of absorption of the pollen antigen. With the average serum and the average subject, 0.5 mg. of pollen injected hypodermically was sufficient to cause a reaction.

For the purpose of the present experiment, one arm of each of 17 nonallergic subjects was sensitized with serial dilutions of serum containing ragweed reagins. Twenty-four or forty-eight hours later pollen was administered orally, either as whole pollen in capsules or in water or in the form of extracts. The doses varied from 15 mg. in the earlier experiments to 5,000 mg. in later trials. When no

9. Bernstein, C., Jr., and Kirsner, J. B.: Oral Pollen Therapy, J. Allergy **8**:221 (March) 1937.

10. Feinberg, S. M., and Bernstein, T. B.: A Method of Measuring Rate and Degree of Absorption of Antigens, J. Allergy **8**:523 (Sept.) 1937.

reactions occurred in the sensitized arm, a subcutaneous injection of 1 cc. of a 1:66 (15 mg.) dilution of the extract was administered, usually on the following day. In some instances the technic was varied by sensitizing the opposite arm with the same serum several days after the oral administration of pollen and by testing the effect of a dose given subcutaneously in both arms.

Comment.—The results are recorded in table 1. After the subcutaneous injection of 15 mg. of pollen, reactions occurred in sensitized areas in all the cases in sites ranging from the fourth to the seventh (serum dilutions of 1:8 to 1:64). With oral administration, all except 1 patient

TABLE 1.—*Results of Oral and Subcutaneous Administration of Pollen to Passively Sensitized Subjects*

Case No.	Ragweed Material Given Orally	Reaction	Highest Site Reacting to 1 Cc. of 1:66 Subcutaneously	Serum Dilution	Factor*
1	1 cc. of 1:66	0	4	1:8	8+
2	1 cc. of 1:66	0	5	1:16	16+
3	5 cc. of 1:66	0	4	1:8	40+
4	2.5 cc. of 1:33	0	5	1:16	80+
5	15 cc. of 1:33	0	5	1:16	480+
6	10 cc. of 1:66	0	Not tested subcutaneously (left hospital)		
	30 cc. of 1:33	0			
7	Pollen, 1 Gm.	1:32	4	1:8	17
8	30 cc. of 1:33	0	6	1:32	1,920+
9	Pollen, 1 Gm.	0	6	1:32	1,920+
10	30 cc. of 1:33	0	6	1:32	1,920+
11	Pollen, 5 Gm.	0	5	1:16	4,800+
12	30 cc. of 1:33	0	7	1:64	3,840+
13	Pollen, 5 Gm.	0	5	1:16	4,800+
14	30 cc. of 1:33	0	7	1:64	3,840+
15	Pollen, 1 Gm.	0	7	1:64	3,840+
16	Pollen, 1 Gm.; 30 cc. of 1:33	0	5	1:16	1,920+
17	Pollen, 1 Gm.; 30 cc. of 1:33	0	Not tested subcutaneously (left hospital)		

* Factor = $\frac{\text{Oral dose} \div \text{highest dilution of reagin}}{\text{Subcutaneous dose} \div \text{highest dilution of reagin}}$

failed to show reactions of the sensitized areas. The subject showing reactions obtained them with the serum dilution as high as 1:32, while with the subcutaneous injection he showed a reaction in the site of injection of the 1:8 dilution. (This subject had a bleeding gastric carcinoma, and it is barely possible that some of the ingested material gained direct entrance to the circulation by way of open vessels.) By dividing the subcutaneous dose by the highest dilution of the reagin at which a reaction was obtained and by comparing the latter figure with the figure obtained in the same way by oral administration, a factor was computed which is recorded in the table for each subject. This factor represents, subject to the experimental conditions, the minimal relative amounts of pollen to be taken orally in order to obtain the same amount of circulating antigen as is obtained by hypodermic administration. The figures range as high as 4,800 times. Since in all instances except 1, no reactions were obtained, the factor may well be above that in some cases.

The criticism may be offered that even though no visible reactions occur at the sensitized sites after oral administration, neutralization or desensitization of those areas may still take place. To settle this question, the following experiments were made on 6 nonallergic subjects: The left arm was sensitized as in previous experiments. Ragweed pollen (maximum dose of 5 Gm.) was given orally. No reactions occurred. Several days later the right arm was sensitized with the same serum. On the following day the standard dose (1 cc. of 1:66 dilution) of pollen was given subcutaneously. In every instance the first reaction (in the left arm) was at least as rapid as that in the right arm, and the height of the reaction was not exceeded in the right arm. This constitutes definite evidence of a lack of invisible desensitization by oral administration.

EXPERIMENTS ON ORAL POLLEN THERAPY

Twenty patients allergic to ragweed were given oral pollen therapy. Of these, 11 had had no pollen therapy prior to 1937, and 8 had had no pollen therapy during 1937. Of the 12 patients who had had hypodermic pollen therapy during 1937, only 1 had had sufficient treatment to lead us to expect satisfactory results. Oral therapy was given after the patient showed symptoms on presenting himself. The duration of treatment was from one to three weeks. In determining the strength of the initial doses we had in mind the relation of the oral to the hypodermic dose, as shown in the experiments described earlier in the paper. One drop of a 1:33 extract of ragweed pollen was regarded as a probably safe initial dose, since it is about 450 times the strength of the first dose (0.05 cc. of a 1:10,000 extract) usually given hypodermically. The doses were given three times daily well diluted. In most instances the maximum dose was 10 to 15 drops at the end of a week. In 2 cases a maximum dose of 30 drops was used.

Comment.—Table 2 indicates the results and other data concerning these patients. In 18 cases no benefit whatever was apparent with regard to the symptoms of hay fever and asthma. In 2 cases (cases 14 and 19) there was moderate improvement. In 1 of these 2 (case 14), while there was some improvement in the hay fever, there was no improvement in the asthma. In other words, there was no amelioration of the asthma in any instance. Attention should be called to the fact that in evaluating results we took into consideration the type of days encountered and the degree of suffering reported by the general group of patients with hay fever. Without such a yardstick, as one of us (S. M. F.) [11] has previously emphasized, therapeutic results in hay fever can hardly be considered seriously.

In 6 cases the pollen extract caused marked gastrointestinal disturbances, consisting mainly of colicky abdominal pain and nausea. One patient failed to follow directions and ingested the extract several times without diluting it. This resulted in sore throat and inability to swallow

11. Feinberg, S. M.: A Method of Evaluation of Results in Hay Fever: Its Application to Certain Modes of Treatment, Ann. Int. Med. **6**:1153 (March) 1933.

TABLE 2.—*Analysis of Data for Twenty Patients Receiving Oral Pollen Therapy*

Case No.	Age	Sex	Diagnosis*	Duration, Years	Hypodermic Pollen Therapy: Prior to 1937	Results	In 1937, Maximum Dose, Pollen Units	Results	Oral Pollen Therapy in 1937 Ragweed Season: Doses, Drops of Ragweed Extract 1:33	Period of Treatment	Improvement	Reactions
1	62	F	HF & A	10	Slight	Partial	700	Poor	1-10	9/11-9/18	None	
2	39	F	HF & A	10	0	.....	0	.....	1-10	9/11-9/18	None	
3	35	F	HF & A	7	0	.....	150	Poor	1-10	8/28-9/11	None	
4	19	M	HF & A	7	1935, 1936	Good	20	Poor	1-30	8/14-9/ 1	None	
5	50	M	HF & A	40	1933, 1934	Fair	40	Poor	1-10	8/28-9/11	None	
6	32	M	HF & A	6	0	.....	20	Poor	1- 5	8/21-8/28	None	5 drops increased hay fever (?)
7	32	F	HF & A	12	1933	Poor	40	Poor	1-10	8/14-8/29	None (intradermal injections helped)	Cramps
8	49	F	HF & A	1	0	.....	0	.....	1- 5	9/ 4-9/18	None	
9	3	M	HF & A	1	0	.....	0	.....	1-10	8/21-9/11	None	
10	57	F	HF & A	17	0	.....	40	Poor	1-15	8/24-8/28	None	Nausea and cramps after 15 drops
11	20	F	HF	11	1936	Good	150	Poor	1-10	8/14-8/24	None	Nausea and cramps
12	44	M	HF & A	1	0	.....	0	.....	1- 5	8/28-9/18	None	
13	43	F	HF & A	9	0	.....	150	Poor	1-10	8/14-8/21	None	
14	45	F	HF & A	3	0	.....	0	.....	2-15	8/14-8/31	Slight for hay fever; none for asthma	
15	25	M	HF & A	16	0	.....	400	Poor	1-10	8/21-8/28	None	
16	37	F	HF & A	3	0	.....	250	Poor	1-10	8/28-9/ 4	None	Nausea and cramps after 3 drops
17	8	M	HF	3	1936	Good	0	.....	1-10	8/21-9/ 4	None	Nausea and cramps
18	34	M	HF	5	1935, 1936	Fair	5,000	Poor	1-30	8/18-9/ 4	None	
19	42	M	HF	8	1933, 1935	Good	0	.....	1-15	8/18-8/28	Moderate	
20	30	M	HF	10	1935	Poor	0	.....	1-10	8/25-9/ 4	None	Nausea and cramps

* HF indicates hay fever; and A, asthma.

food for several hours subsequently. Apparently there was a local reaction in the esophagus. One patient thought that the oral therapy increased his hay fever. Any possible increase in hay fever symptoms could not be definitely ascertained in this case or in the others because of the previous existence of active symptoms. To determine this effect, preseasonal oral therapy will probably have to be tried.

COMMENT

It may be argued that the doses used were not therapeutically adequate. We are inclined to believe that such is the case, in view of our experimental results and since in the group of patients with hay fever absorption of the antigen may be even less than in the nonallergic subjects. However, there is a definite obstacle to the use of much larger doses, i. e., the tendency for marked local reaction in the gastrointestinal tract. Furthermore, the variability of enteral absorption tends to lead occasionally to marked overdosage. Criticism can be leveled at our experimental work because it was done on nonallergic subjects. It should be remembered, however, that in other experiments in the past it has been found that the allergic subject shows even more diminished absorption than the nonallergic subject. Again, our previous experiments [10] and present control experiments on hypodermic absorption were done on nonallergic subjects.

Perhaps a serious objection to our report is that all the therapeutic work was done coseasonally. We admit that with a long-continued preseasonal method of oral administration of pollen the results might have been different. We believe, however, that our work compares favorably with that of others, since coseasonal therapy was studied in most of them. Our lack of successful results may possibly be due to the difficulties encountered in desensitizing patients with ragweed sensitivity as compared with those encountered in desensitizing patients with other types of pollinosis. We realize that our group of cases is a small one, but we believe that the close study of the patients, coupled with the experimental work on absorption, makes the interpretation of our results of significance.

The disadvantages of hypodermic therapy, to which attention is called earlier in the paper, seem to apply almost as well to oral therapy. From our experience we doubt whether the average sufferer from hay fever would consider the discomfort of a gastrointestinal reaction less than that of the hypodermic needle. The total cost of frequently administered large doses orally would probably be greater than the physician's fee for a course of hypodermic injections. The question of constitutional reactions cannot be determined at this time, and we therefore cannot argue that point.

SUMMARY

A group of 20 patients with ragweed hay fever and asthma were given oral doses of pollen extracts, beginning with 1 drop of a 1:33 dilution and reaching a maximum of 10 to 30 drops three times daily. Eighteen were not benefited and 2 obtained moderate benefit. Gastrointestinal complaints were common. Experiments on absorption of ragweed pollen or extract administered orally to nonallergic subjects indicate that the amount of the antigen reaching the circulation in a unit of time is not more than about one four thousandth of the amount demonstrated in the circulation after hypodermic administration. We are justified in the conclusion that in ragweed hay fever in the Middle West, coseasonal oral pollen therapy is of little value.

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Progress in Internal Medicine

INFECTIOUS DISEASES

REVIEW OF CURRENT LITERATURE

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During the past year much new information was gathered concerning influenza, streptococci, chemotherapy and a number of "new" and rare diseases. The program for the control of pneumonia has widened so that many states have organized commissions and committees for the study and control of pneumonia and a national committee has been appointed by the surgeon general. It is fortunate that concerted studies are now being made on some of the most common of all human ills, namely, infections of the respiratory tract. Whatever success attends the efforts in prevention or control of colds, catarrh and influenza will automatically reduce the incidence of pneumonia, for in most cases pneumonia is preceded by a mild infection of the respiratory tract.

INFLUENZA

A report of important studies on influenza by English investigators is embodied in a monograph published by the British Medical Research Council.[1] The clinical description of influenza in patients proved to harbor the virus now believed to be the cause of the disease is similar in most respects to the description of the disease as it occurred in pandemic form in 1918-1919. A few differences were noted in recent epidemics: (1) symptoms of profound mental depression were absent, (2) relative bradycardia was not found often, (3) convalescence was rapid and (4) the leukocyte count was usually normal or slightly increased and leukopenia was uncommon. The erythrocyte sedimentation rate was seldom increased in cases of uncomplicated influenza.

Influenza manifests itself with varying degrees of severity, which may be listed in order as follows: (*a*) subclinical infections without symptoms, (*b*) "simple" influenza, (*c*) influenza with bronchiolitis and (*d*) influenza pneumonia. Although influenza can be diagnosed with

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1. Stuart-Harris, C. H.; Andrewes, C. H.; Smith, W.; Chalmers, D. K. M.; Cowen, E. G. H., and Hughes, D. L.: A Study of Epidemic Influenza, with Special Reference to the 1936-1937 Epidemic, Medical Research Council, Special Report Series, no. 228, London, His Majesty's Stationery Office, 1938.

certainty only when the virus is recovered, the authors attempt to separate influenza from other infections of the respiratory tract, such as "febrile catarrh," simple coryza, pharyngitis and streptococcic tonsillitis, and give the following summary of features to assist in a clinical differentiation:

I. EPIDEMIC INFLUENZA

1. *History.*—Onset is sudden without premonitory symptoms. The first symptoms are general or constitutional, comprising headache, shivering, muscular pains and dizziness. Respiratory symptoms develop later with coryza, sore throat and cough.

2. *Course of the fever.*—Rapid rise of temperature on the second day of illness may or may not be followed by a remission of temperature on the third day, but there is usually renewed pyrexia on the fourth day. General symptoms continue to dominate the illness but a short cough with but slight expectoration is more prominent during the later stages.

3. *General aspect.*—The typical facies is heavy and drowsy with drooping eyelids, glistening eyes, dusky facial flush and slightly cyanosed lips.

4. *Physical signs.*—These are, obstructed nose, furred tongue, husky but not hoarse voice, the signs of pharyngitis. The particular characteristics of the pharyngitis are its posterior position, its large-vessel injection, a tendency to dryness, and a granular appearance. Signs in the chest comprise rhonchi or a few râles at the bases towards the end of the fever.

5. *Complications.*—Chest complications predominate over all others with a characteristic picture of "bronchiolitis," and a range of pneumonic conditions also characteristic clinically. Pneumococcal complications are common. Sinusitis occurs chiefly as a late sequela.

6. *Variations.*—On the whole cases are remarkably uniform in clinical appearance and there is no tendency to admixture with other diseases such as tonsillitis. The most important variation from the typical case is the case with chest complications.

7. *Characteristics of epidemic.*—The epidemic appears suddenly, rises rapidly to a peak and disappears within about 2 weeks if the population is a closed one.

II. "FEBRILE CATARRHS"

In contrast with the features described above as characteristic of epidemic influenza, the catarrhal conditions which were studied in the Woolwich and Chatham (November) epidemics showed the following characteristics:

1. *History.*—Onset is insidious with premonitory "cold" and cough for several days. Respiratory symptoms usher in the disease and sore throat and cough dominate the picture. Cough is paroxysmal, irritating and painful, with substernal soreness over the trachea. Expectoration varies greatly, being sometimes profuse. Hoarseness of the voice develops.

2. *Course.*—General symptoms of illness are overshadowed at the onset but are present during the fever with headache and muscular pains. The fever has no characteristic course and shows no special tendency to be diphasic in type.

3. *General aspect.*—Often that of a patient with a heavy cold, or with brightly flushed face, injected conjunctivae and slightly cyanosed lips.

4. *Physical signs.*—These are obstructed nose, clean or furred tongue, hoarse voice, and signs of tonsillitis or pharyngitis. Signs of tonsillitis are involvement

of the anterior as well as the posterior part of the fauces, intense capillary injection, and exudation of mucous, mucopurulent or follicular material. Signs in the chest are absent usually but rhonchi may be heard.

5. *Complications.*—Chest complications are commoner than others and comprise bronchitis of large or small tubes, or bronchopneumonia. The haemolytic streptococcus is a common incitant of the chest complications.

6. *Variations.*—The clinical picture varies greatly with alternation of the pharyngo-laryngo-tracheitis syndrome and frank tonsillitis, liable to be confused with follicular streptococcal tonsillitis.

7. *Characteristics of epidemic.*—Gradual development from the basal respiratory disease of the population (coryza and tonsillitis). Slow rise and fall with prolonged duration of epidemic over several weeks.

In tabular form the essential clinical features are contrasted as follows: [2]

	Epidemic Influenza	Febrile Catarrhs
Onset	Sudden	Insidious
Symptoms	Constitutional symptoms predominate	Respiratory symptoms predominate
Cough	Short and dry	Paroxysmal, irritating, painful, often productive
Voice	Husky	Hoarse
Throat	Posterior pharyngitis; no exudate	Tonsillitis as well as pharyngitis; exudate common
Fever	Sometimes diphasic	Rarely diphasic
Complications	Bronchiolitis and pneumonia	Bronchitis or bronchopneumonia
Epidemic	Short with rapid "peaking"	Prolonged and "grumbling"
Contacts	Clinical picture uniform, although graded in severity	Clinical picture variable, with frank tonsillitis in contacts
Leukocyte count	Not diagnostic	Not diagnostic
Virus	Influenza virus recoverable from pharynx	Influenza virus not concerned

The monograph contains a detailed report of experiments with the use of vaccine and of specific immune serum in the prevention and treatment of influenza. Vaccine increased the specific immune bodies in the blood strikingly, even to some extent against influenza viruses slightly different serologically from the strain used as the antigen. The experiments are also described in a separate publication by Andrewes.[3] He asserts that contrary to much opinion, a virus vaccine killed with formaldehyde is as effective an antigen as a living one. Difficulty was encountered in preparing a vaccine free from undesirable protein material. He vaccinated several groups of volunteers with virus inactivated with solution of formaldehyde and succeeded in increasing the antibodies against influenza in the blood. The antibodies reached their height in about a fortnight. It was disappointing that in several persons thus vaccinated influenza developed, which indicates that the vac-

2. Stuart-Harris, C. H.: Epidemic Influenza, Brit. M. J. **2**:516-518 (Sept. 11) 1937.

3. Andrewes, C. H.: Influenza: Four Years' Progress, Brit. M. J. **2**:513-515 (Sept. 11) 1937.

cine is not as yet reliable. The results obtained by treating patients with immune horse serum were not convincing, but the severity of the infection in mice was definitely lessened even if the serum was injected on the first or the second day of the disease.

Francis and his co-workers [4] studied an epidemic of disease of the respiratory tract in the winter of 1936-1937. The washings from the throat in 52 of 100 cases contained the virus of epidemic influenza. In 48 cases a diagnosis of influenza was made on the basis of neutralization tests and the complement fixation reaction. By using the same procedures, numbers of patients with infection of the respiratory tract were found during the epidemic whose disease was caused by some agent other than the influenza virus. The authors emphasize the importance of persons who have mild or subclinical infections as disseminators of infection. Mild or subclinical infections probably account for the frequency with which evidence of immunity is found in the apparent absence of a preceding attack of influenza.

Two British investigators [5] were successful in vaccinating mice against influenza by using heat-killed suspensions of "elementary bodies" of influenza virus. These bodies were obtained by differential centrifugation and were believed by the authors to contain the immunogenic fraction of influenza virus. Less success attended the immunization of ferrets, but since large doses of viruses were necessary to infect these animals the authors are still optimistic about the success of the vaccine if given to human beings.

Neal and Wilcox [6] studied a number of patients who supposedly had influenza and in whom encephalitis later developed to find whether the two conditions were etiologically related. In several cases no evidence was found to confirm a diagnosis of influenza. It is also known that animals experimentally infected with influenza virus do not show nervous symptoms, and in many outbreaks of influenza, encephalitis does not occur; both facts fail to support any relation between the two. It is unknown whether certain acute infections of the respiratory tract permit the invasion of an agent which causes encephalitis or whether the first symptoms of encephalitis itself are manifested in the respiratory tract. Hamburger [7] discusses various opinions concerning the effect

4. Francis, T.; Magill, T. P.; Rickard, E. R., and Beck, M. D.: Etiological and Serological Studies in Epidemic Influenza, Am. J. Pub. Health **27**:1141-1159 (Nov.) 1937.

5. Fairbrother, R. W., and Hoyle, L.: Active Immunization Against Experimental Influenza: Use of Heat-Killed Elementary Body Suspensions, Brit. J. Exper. Path. **18**:430-435 (Dec.) 1937.

6. Neal, J. B., and Wilcox, H. L.: Does the Virus of Influenza Cause Neurological Manifestations? Science **86**:267-268 (Sept. 17) 1937.

7. Hamburger, W. W.: The Heart in Influenza, M. Clin. North America **22**:111-121 (Jan.) 1938.

of influenza on the heart. It seems that influenza is not a frequent cause of heart disease, yet in a certain number of patients evidence of disorder of the circulatory system is present.

The detection of an epizootic virus disease of ferrets by Slanetz and Smetana [8] must serve as a warning to those who employ these animals for the diagnosis of influenza. The clinical course of the infection resembles the disease caused by the inoculation of ferrets with human influenza virus, but there seems to be no immunologic relation between the viruses of ferret disease, of human influenza and of canine distemper.

Influenza Pneumonia.—Several studies based on the newly discovered virus of influenza seem to clarify a number of problems concerning influenza pneumonia. Straub [9] studied the lungs of mice infected with the virus and found necrobiosis and fibroid necrosis of the epithelium of the respiratory and terminal bronchioles leading to complete desquamation. There were secondary dilation of the bronchioles and collapse of the alveoli, with edema and hyperemia. The cellular reaction was chiefly of the mononuclear type of cell. The lesions as described were strikingly like those reported by MacCallum, Goodpasture and others in human beings in the 1918-1919 pandemic, at which time a number of observers suggested that a virus may be operative. Snow and Cassasa [10] note the frequency with which emphysema and atelectasis occur in human beings with influenza. Scadding [11] reports a study of 58 patients with influenza. Of these patients, 18 gave no evidence of pulmonary involvement, in 21 there were physical signs of congestion and in 19 there was evidence of consolidation. Seven of the last-mentioned group died. Bacteriologic study of the sputum showed no special variety of bacteria to be present. Pneumococci generally predominated in the patients who had pneumonia. In 1 case the staphylococcus alone was operative. Scadding believes that the findings in his cases were essentially the same as those reported in 1918-1919.

It seems that heretofore three forms of pulmonary infection have been called influenza pneumonia: The first is caused by the virus of influenza itself and perhaps represents the most severe form of the disease, the second is caused by the influenza bacillus of Pfeiffer and the third is caused by a variety of bacteria, either as single species

8. Slanetz, C. A., and Smetana, H.: An Epizootic Disease of Ferrets Caused by a Filterable Virus, J. Exper. Med. **66**:653-666 (Dec.) 1937.

9. Straub, M.: The Microscopical Changes in the Lungs of Mice Infected with Influenza Virus, J. Path. & Bact. **45**:75-78 (July) 1937.

10. Snow, W., and Cassasa, C. S. B.: Obstructive Emphysema and Atelectasis in Influenza, J. A. M. A. **109**:1886-1888 (Dec. 4) 1937.

11. Scadding, J. G.: Lung Changes in Influenza, Quart. J. Med. **6**:425-466 (Oct.) 1937.

or in mixtures, invasion being made possible by the harmful action of the virus of influenza on the lung or by the shocklike reaction in cases of severe influenza. Pneumonia in influenza may therefore be caused in at least two ways: First, a specific lesion caused by influenza virus itself may be secondarily invaded by other bacteria; second, pneumonia may occur as the result of severe systemic infection with circulatory failure and a shocklike condition during which the lungs are susceptible to infection with any or all of a mixture of bacteria which happen to be present in the respiratory tract. Recent studies have suggested a similar state of affairs regarding the virus diseases measles, vaccinia and psittacosis.

Haemophilus Influenzae Meningitis.—Fothergill [12] reports slight success in the treatment of meningitis caused by Pfeiffer's bacillus with specific immune serum. The mortality of untreated patients is nearly 100 per cent; it appeared to be reduced to 84 per cent by serum treatment. In the years between 1933 and 1936 the following specific forms of meningitis were encountered among children.

Organism	No. of Cases
Haemophilus influenzae	51
Pneumococcus	38
Streptococcus haemolyticus	36
Tubercle bacillus	31
Meningococcus	27
Others	38

The incidence of the influenza bacillus as a cause of meningitis is surprisingly large.

PNEUMONIA AND THE PNEUMOCOCCUS

Perhaps the most important development in the field of pneumonia during the past year is the organization of committees or commissions in many states and cities for its study and control and finally the appointment of a national committee by the surgeon general. As pointed out by Cole [13] in a recent paper, physicians acting individually or in small groups have not been effective in controlling such epidemic diseases as tuberculosis, diphtheria and smallpox unless assisted by public health organizations. To popularize new methods for the control or treatment of pneumonia or of any epidemic disease, it is necessary to provide for the instruction of physicians generally, particularly of those who have been in practice for many years. In addition, the difficulties

12. Fothergill, L. D.: Hemophilus Influenzae (Pfeiffer Bacillus) Meningitis and Its Specific Treatment, New England J. Med. **216**:587-590 (April 8) 1937.

13. Cole, R.: Possibilities for Pneumonia Control as Indicated by Present Scientific Knowledge, Mil. Surgeon **81**:241-255 (Oct.) 1937.

of typing pneumococci in sputum must be minimized by the establishment of reliable laboratories in convenient places; physicians must be taught when to use antipneumococcus serum, how to obtain it and how to use it. These services can best be rendered by public health agencies. In many states typing of pneumococci is performed free, and serum is supplied free to those patients who cannot afford to buy it or to those who find it a hardship to pay for it.

Further report is made on the treatment of pneumococcic pneumonia with unconcentrated rabbit serum at the Hospital of the Rockefeller Institute.[14] Among 54 patients with pneumonia caused by 8 types of pneumococci, I, II, V, VI, VII, VIII, XIV and XVIII, only 2 deaths occurred, a mortality rate of 3.7 per cent. One of the striking features noted was the rapid recovery of most patients; the average interval from the beginning of treatment with serum until the disappearance of signs of acute illness was only twenty-seven hours. In 18 cases in which the full therapeutic dose was given in a single injection, the interval between the beginning of therapy and the crisis was about eight hours. Good results were not obtained from the serum treatment of 13 patients with pneumonia due to type III pneumococci. Six of these patients died.

An unexpected disadvantage in the use of type XIV antipneumococcus horse serum was discovered by Finland and Curnen.[15] Because serious and fatal reactions were noted to occur in patients treated with serum, attempts were made to find the reason. It was discovered that each of the different samples of type XIV antipneumococcus serum tested agglutinated human erythrocytes of all four blood groups. Other types of antipneumococcus horse serum were also tested, but only 2 agglutinated erythrocytes in low dilution. When type XIV antiserum was absorbed with type XIV pneumococci, agglutinins for the pneumococci and for the erythrocytes were removed; absorption with erythrocytes removed agglutinins for erythrocytes but not for the pneumococci. A number of antipneumococcus rabbit serums prepared with the same strains of type XIV pneumococci used in preparing horse serum were tested, but none agglutinated human erythrocytes in high titer.

Attempts were made[16] to test the effect of high temperature in the treatment of infection due to type III pneumococci. Previous experi-

14. Horsfall, F. L.; Goodner, K., and MacLeod, C. M.: Antipneumococcus Rabbit Serum as a Therapeutic Agent in Lobar Pneumonia, New York State J. Med. **38**:245-255 (Feb. 15) 1938.

15. Finland, M., and Curnen, E. C.: Agglutinins for Human Erythrocytes in Type XIV Antipneumococcus Horse Serums, Science **87**:417-418 (May 6) 1938.

16. Shaffer, M. F.; Enders, J. F., and Wilson, J.: The Effect of Artificial Fever and Specific Antiserum on the Organisms Present in Cases of Type III Pneumococcus Meningitis, J. Clin. Investigation **17**:133-145 (March) 1938.

ments with rabbits had led to the belief that they are immune to infection with certain strains of type III pneumococci which fail to grow at a temperature over 105 F. because the temperatures of rabbits after infection often exceeded this level. Accordingly, the body temperature of 2 patients with meningitis due to type III pneumococci was raised artificially to 105 F. or higher for several hours. After this treatment the number of pneumococci in the spinal fluid was markedly reduced, but in neither case was the fluid sterilized. Two other patients were given injections of type III antipneumococcus rabbit serum intraspinally. Temporary sterility was accomplished in both cases, but pneumococci later reappeared. In 1 case, combined fever therapy and antipneumococcus serum failed to prevent death.

In experimental studies of pneumococcic pneumonia in dogs, Robertson [17] made further important contributions in regard to the problem of immunity. Dogs were actively and passively immunized by the injection of large amounts of specific antipneumococcus horse serum and by vaccination with living and dead pneumococci. Neither the passive nor the active immunity thus produced served to protect the dogs regularly against pneumonia when pneumococci were injected deep into the lungs according to the author's method. The resulting lesion in the lung was often less in extent, and the disease produced was of shorter duration, than that in immunized dogs. The combination of active and passive immunization was better than one or the other alone, but the greatest protection was attained only when the infective dose of pneumococci and the specific immune serum were injected at the same time. The experiments are not encouraging as far as the ultimate practical application of vaccine prophylaxis against pneumonia in man is concerned.

Studies are constantly under way to test the efficiency of different forms of treatment for pneumonia, notably by diathermy and roentgen irradiation. In regard to diathermy, Coulter [18] states that there is no evidence to show its effectiveness in treating patients with pneumonia except as a counterirritant to relieve pleuritic pain. Another paper [19] deals with roentgen therapy of lobar pneumonia. The results described are unconvincing and cannot be accepted as showing the value of this

17. Robertson, O. H.: The Effect of Increased Antipneumococcal Immunity on the Inception of Experimental Lobar Pneumonia in the Dog, J. Exper. Med. **66**:705-727 (Dec.) 1937.

18. Coulter, J. S.: Medical Diathermy in Pneumonia, M. Clin. North America **22**:61-68 (Jan.) 1938.

19. Powell, E. V.: Roentgen Therapy of Lobar Pneumonia, J. A. M. A. **110**:19-22 (Jan. 1) 1938.

form of treatment. Whenever an author reports a mortality rate of from 2.5 to 4 per cent in a large number of cases of pneumonia after the first day or two of treatment by any method, two conclusions are possible: first, that a marvelous new remedy is at hand or, second, that the diagnoses are not in accord with those of most clinicians, since practically all studies have shown that the mortality rate for patients with pneumonia untreated with specific immune serum varies between 30 and 40 per cent. Accurate experimental studies on the effect of roentgen rays on pneumococcic infections are urgently needed.

A new form of vaccine against pneumococcic pneumonia prepared with fractions of the pneumococcus body is being tested on a large scale by Felton. Thousands of persons have been vaccinated, and other thousands under similar environmental conditions are being observed as controls. The studies made thus far are not sufficiently advanced to permit a statement of the effect obtained by vaccination in preventing pneumonia.

Lindqvist [20] studied the vitamin A content of the serum of patients with pneumonia. Carotenoid and vitamin A were low in amount during the disease, but during convalescence the amount increased rapidly without the addition of the vitamin to the diet. During fever large quantities escaped in the urine. One week after the crisis the vitamin content was three times as high as it was at the lowest point. There is no evidence to show that the administration of vitamin A during pneumonia has any effect on the disease.

Dick and Boor [21] report the isolation of a toxin or toxin-like substance produced by autolysis of pneumococci which seems to produce a weak antitoxin. It is questionable if this substance is not the same as the purpura-producing substance obtained by autolysis of pneumococci, as reported by many other investigators.

Because most deaths of children with nephrosis are caused by pneumococcic peritonitis, MacLeod and Farr [22] studied the relationship of types of pneumococci carried in the nasopharynx of children with nephrosis to determine whether the infection was exogenous (contracted from outside sources) or endogenous (arising from the invasion of bacteria carried in the nasopharynx). In each of 6 children studied, the same type of pneumococcus was recovered from the throat as from

20. Lindqvist, T.: Untersuchungen über das Vitamin A bei Pneumonie, Klin. Wchnschr. **16**:1345-1347 (Sept. 25) 1937.

21. Dick, G. F., and Boor, A. K.: Pneumococcus Toxin and Antitoxin, J. Infect. Dis. **61**:228-233 (Sept.-Oct.) 1937.

22. MacLeod, C. M., and Farr, L. E.: Relation of the Carrier State to Pneumococcal Peritonitis in Young Children with the Nephrotic Syndrome, Proc. Soc. Exper. Biol. & Med. **37**:556-557 (Dec.) 1937.

the pus in the abdomen, suggesting that invasion occurred with the types habitually present. Types VI, X, XIX, XX and XXVIII were found.

Pickrell,[23] in following up previous work of Stillman and others, tested the effect of alcohol in reducing the resistance of rabbits to pneumococcic infection. Alcoholic intoxication was found to destroy resistance to pneumococci even in animals rendered highly immune by vaccination. The loss of resistance was presumably due to inhibition of the vascular inflammatory response as a result of intoxication. Leukocytic immigration to the site of infection was negligible, and the bacteria grew unhampered. Ether anesthesia had a similar inhibitory effect on the inflammatory response.

Moon [23a] studied the origin and pathology of what is perhaps the most common form of pneumonia, namely, that form called terminal pneumonia. Congestion, stasis and edema, which, he believes, constitute the essential pathologic features of shock, are the chief factors in the causation of this form of pneumonia.

The publication of White's [24] review dealing with practically all the published papers concerning the biology of the pneumococcus must be regarded as a notable achievement. In this comprehensive and critical survey, 1,593 papers are surveyed, and the subject is brought together in a clear, logical and unified treatise which for many years should be a source of stimulation for those interested in infectious disease and pneumonia. The pages fairly bristle with suggestions for attack on unsolved problems. It is of interest to speculate on the amount of time which has thus far been devoted to research on the pneumococcus. If, for example, each of the papers listed represents an average of three months of work, one could hazard an estimate that about four hundred years has been spent on the investigation of this amazing cell.

STREPTOCOCCI AND STREPTOCOCCIC INFECTIONS

Bradley [25] made a study concerning the epidemiology of infections due to hemolytic streptococci. About 20 per cent of a group of patients in one hospital were found to be carriers of hemolytic streptococci,

23. Pickrell, K. L.: Effect of Alcoholic Intoxication and Ether Anesthesia on Resistance to Pneumococcal Infection, Proc. Soc. Exper. Biol. & Med. **38**: 265-267 (March) 1938.

23a. Moon, V. H.: Origin and Pathology of Common Terminal Pneumonia, Arch. Path. **26**:132-143 (July) 1938.

24. White, B.; Robinson, E. S., and Barnes, L. A.: The Biology of Pneumococcus, New York, Commonwealth Fund, Division of Publications, 1938.

25. Bradley, W. H.: Epidemiology of Streptococcal Infections, Guy's Hosp. Rep. **87**:372-390 (July) 1937.

most of which, according to Griffith's classification, were of the types potentially pathogenic for man. The distribution of types was as follows:

Type	No. of Cases	Type	No. of Cases	Type	No. of Cases
I	3	IX	3	XVIII	4
II	2	X	1	XXI	7
III	1	XI	3	XXII	11
IV	16	XIII	9	XXV	1
V	7	XV	3	XXVI	1
VI	1	XVI	5	XXVII	1
VII	1	XVII	1	XXVIII	3

Most of the patients examined were transient carriers, usually for only a few days, but a number of them persistently carried the streptococci. In several examples of epidemics of hemolytic streptococcic infections in families, a single type was involved and was sometimes found in all exposed persons. Overcrowding and uncleanliness favored the appearance of many carriers in a group. The author regards acute tonsillitis due to hemolytic streptococci as a dangerous infection which equals diphtheria in epidemiologic significance. It is perhaps not generally realized that bacteria in the dry state may float about in the air much as does tobacco smoke (Wells).

Shaw [26] also studied the types of hemolytic streptococci found in patients. Streptococci of Lancefield's group A were present in 90 per cent of infections in human beings; in 1 case, a streptococcus of group C was present and in another a streptococcus of group G. Type II and type XXV streptococci were most frequently found in cases of puerperal infection; types IV, VI, VIII and XXII were also often present. Among 29 strains of streptococci found in the nasopharynges of normal persons, 9 were of group A, 1 was of group B and 1 was of group C. Eighteen of the strains failed to form soluble hemolysin and could not be classified.

Smith and Sherman [27] studied 109 cultures of hemolytic streptococci isolated from 45 samples of human feces. Streptococci of Lancefield's group A were not encountered. The streptococci most commonly found were those of group D and a related "enterococcus," Streptococcus durans. Members of groups B, C, F and G were also found.

Long and Bliss [28] studied the incidence of minute beta hemolytic streptococci and ordinary beta hemolytic streptococci in the flora of the throats of healthy and of diseased persons. The incidences of the two varieties in healthy persons were about the same (12 per cent),

26. Shaw, C.: Serological Group and Typing of Hemolytic Streptococci, Lancet **2**:1193-1194 (Nov. 20) 1937.

27. Smith, F. R., and Sherman, J. M.: The Hemolytic Streptococci of Human Feces, J. Infect. Dis. **62**:186-189 (March-April) 1938.

28. Long, P. H., and Bliss, E. A.: Studies upon Minute Hemolytic Streptococci, J. Infect. Dis. **62**:52-57 (Jan.-Feb.) 1938.

but when cultures were made repeatedly, the incidence of the ordinary type increased considerably more than that for the minute forms. In persons with rhinitis or infections of the upper respiratory tract the incidence of both forms of streptococci was lower than usual. Children whose tonsils had not been removed "showed a definitely higher subject incidence for both groups" of streptococci. The minute beta hemolytic streptococcus was seldom found in patients with chronic disease, scarlet fever or streptococcic tonsillitis. Minute forms, however, were present in 55 per cent of the patients with rheumatic fever or its sequelae and in 96 per cent of 48 patients with diffuse glomerular nephritis.

In experimental studies,[29] hemolytic streptococci were injected into the paranasal sinuses of cats and rabbits and were traced to their deposition in the lungs. The fact that streptococci were found in the lungs, liver and spleen indicates that the anatomic pathway from the sinuses must be through the paralaryngeal and paratracheal lymph nodes and lymph vessels to the great veins which empty into the right auricle and that from there the organisms are carried by the blood to the lungs and elsewhere. The passage of infection from the sinuses by way of direct lymphatic connection to the lungs is rather unlikely, since the lymphatic chains are frequently interrupted by lymph nodes.

Dawson and his associates [30] report the existence of a scheme of bacterial variation among hemolytic streptococci which closely resembles the well known M, S and R forms of pneumococci and other bacteria. The capsular polysaccharide substance of the mucoid (M) forms of group A streptococci, unlike that of the pneumococcus, is not type specific.

After an uncritical and poorly controlled study Rosenow and Heilman [31] report favorable results in the prevention and treatment of colds and influenza by the use of vaccines made from a mixture of 400 strains of streptococci. No attempt was made to differentiate the cold from influenza, and no mention is made of the recent studies on the viruses of either disease or of the fact that numerous bacteria other than streptococci may secondarily invade persons ill with colds or influenza. In spite of a statement to the contrary, there is no evidence that vaccines of any kind favorably influence in a specific manner subsequent infections with streptococci. The use of vaccines in the specific treatment of any infection is illogical. One is amazed at the persistence of some

29. Larsell, O.; Veazie, L., and Fenton, R. A.: Streptococcus Infection of the Lung from the Paranasal Sinuses, Arch. Otolaryng. **27**:143-150 (Feb.) 1938.

30. Dawson, M. H.; Hobby, G. L., and Olmstead, M.: Variation in the Hemolytic Streptococci, J. Infect. Dis. **62**:138-168 (March-April) 1938.

31. Rosenow, E. C., and Heilman, F. R.: Streptococcal Vaccines in the Prevention and Treatment of Respiratory Infections, Am. J. Clin. Path. **8**:17-27 (Jan.) 1938.

investigators who continue to publish work so consistently at odds with the bulk of evidence, with little or no reference to work which fails to corroborate their results. If such publications did not confuse and obstruct scientific thought, they might be regarded as stimulating or at least provocative, but unfortunately they are for the most part simply misleading. There seems to be no end to the number of diverse diseases which Rosenow believes to be caused by streptococci, as indicated in the following list:

Influenza	Epidemic hiccup	Ocular diseases
Colds	Erythema nodosum	Prostatitis
Poliomyelitis	Renal stones	Myositis
Encephalitis	Chorea	Myasthenia gravis
Multiple sclerosis	Arthritis	
Herpes zoster	Rheumatic fever	
Neuritis	Epidemic gastroenteritis	
Torticollis	Appendicitis	

Schottmüller, discussing a paper read by Rosenow at the German Congress of Medicine at Wiesbaden, said that Rosenow's theory of elective localization not only must be disregarded but must be combated. Similar views were expressed by Pette.

SULFANILAMIDE

It is not within the province of this review to list all the papers published during the past year on the subject of sulfanilamide. A great many papers are reports on single patients treated with the drug, and many studies are not scientifically controlled. General reviews may be found in the papers of Long and Bliss [32] and others.

Mode of Action.—In Bliss and Long's [33] experiments, sulfanilamide apparently did not kill bacteria directly but seemed to inhibit the growth of hemolytic streptococci and Clostridium welchii both in vitro and in vivo. Temporary inhibition of growth or bacteriostasis seemed to permit the natural forces of the body to cope with the infection successfully and led to recovery. The authors tried to show the importance of the phagocytic action of the polymorphonuclear leukocytes as one of the natural forces. They injected benzene into mice, according to Rich's technic, to destroy the leukocytes before infecting the mice with streptococci and treating them with sulfanilamide. Because the mice rendered granulocytopenic died and controls recovered, the authors assume that polymorphonuclear leukocytes are required in the process of recovery. While this may be true, the experiments are open to criticism, since

32. Long, P. H., and Bliss, E. A.: The Clinical Use of Sulfanilamide and Its Derivatives in the Treatment of Infectious Disease, Ann. Int. Med. **11**:575-592 (Oct.) 1937.

33. Bliss, E. A., and Long, P. H.: Observations on the Mode of Action of Sulfanilamide, J. A. M. A. **109**:1524-1527 (Nov. 6) 1937.

poisoning a mouse with benzene causes other changes in addition to reducing the number of circulating leukocytes.

According to Gay and Clark,[34] the serum of rabbits treated with sulfanilamide inhibited the growth of streptococci in vitro. Neter[35] reports similar bacteriostatic effects of the drug on the meningococci present in the spinal fluid of patients with meningitis.

Finkelstein and Birkeland[36] conclude that the presence of serum is necessary for the drug to be effective in causing phagocytosis in vitro; the serum-sulfanilamide complex acts, therefore, as an opsonin. There seems to be no reason to confuse the matter by introducing the word opsonin. Obviously, when any drug is used to inhibit growth of bacteria, some of the bacteria will die and be taken in by phagocytes.

Osgood[37] and Brownlee could not demonstrate any direct effect of sulfanilamide on phagocytosis. The drug apparently decreases the rate of division of streptococci, and its major effect seemed to the authors to be the neutralization of toxins. Their conclusion is not substantiated by evidence. Huntington[38] was unable to show that sulfanilamide had any effect on fibrinolysis or on the formation of toxin in vivo. It also failed to inactivate small amounts of toxin.

Streptococcic Infections.—Sulfanilamide exerts its beneficial effect on infections caused by hemolytic streptococci and not on those caused by Streptococcus viridans. Spink at a recent meeting reported long remissions in several cases of subacute bacterial endocarditis after treatment with sulfanilamide. Mellon and Cooper[39] call attention to certain strains of group A hemolytic streptococci which may at times cause green pigmentation on blood agar plates and may be mistaken for Str. viridans. The strains they studied grew in colonies surrounded by a broad grass-green zone when plated on blood agar plates at a p_H of 6, but when the same strain of streptococci was plated on blood agar at p_H 8, the hemolytic activity became dominant, and the colonies were surrounded by zones of hemolysis. The production of hemolysis or of

34. Gay, F. P., and Clark, A. R.: Mode of Action of Sulfanilamide in Experimental Streptococcic Empyema, J. Exper. Med. **66**:535-548 (Nov.) 1937.

35. Neter, E.: Bacteriostatic Action of Sulfanilamide upon Meningococcus in Spinal Fluid, Proc. Soc. Exper. Biol. & Med. **38**:37-40 (Feb.) 1938.

36. Finkelstein, R., and Birkeland, J. M.: The Mode of Action of Sulfanilamide and Prontosil, Science **87**:441-442 (May 7) 1938.

37. Osgood, E. E.: Culture of Human Marrow: Studies on the Mode of Action of Sulfanilamide, J. A. M. A. **110**:349-356 (Jan. 29) 1938.

38. Huntington, R. N.: Failure of Sulfanilamide to Prevent Hemolysis, Fibrinolysis and Production of Erythrogenic Toxin by Hemolytic Streptococci in Vitro, Proc. Soc. Exper. Biol. & Med. **38**:328-331 (April) 1938.

39. Mellon, R. R., and Cooper, F. B.: The Bi-Phasic Nature of Certain Alpha-Prime (?) Hemolytic Streptococci, Proc. Soc. Exper. Biol. & Med. **38**: 158-160 (Feb.) 1938.

green coloration of blood agar by certain strains of hemolytic streptococci therefore depends on the environment in which the bacteria grow. If these streptococci obtained from patients successfully treated with sulfanilamide happen to be plated on mediums favoring the development of the green phase, one is likely to be misled into ascribing a curative effect of the drug for infection due to Str. viridans.

One also must not be misled by overenthusiastic and careless statements prepared for general readers concerning the value of various therapeutic measures, especially when they are printed in journals otherwise noted for their accuracy. In one such article [40] streptococcic meningitis untreated with sulfanilamide was said to be invariably fatal. This is not true, for several reports of recovery of patients not treated with the drug have appeared within the past year.

A number of observers [41] have obtained some degree of success in treating erysipelas with sulfanilamide, but here again, one must be cautious in accepting such reports as final when one recalls the exceptionally good results claimed for the treatment of this disease with immune serum, with roentgen rays and with ultraviolet radiation. Colebrook,[42] in one of his later papers, continues to write of satisfactory results in treating puerperal sepsis caused by hemolytic streptococci with sulfanilamide but claims that the resolution of the process seems to be less spectacular than it appeared to be in the earliest days of enthusiasm.

It was disappointing to read of the apparent ineffectiveness of sulfanilamide in the treatment of scarlet fever in Hogarth's [43] paper. The failure may raise some question of the actual etiologic role of hemolytic streptococci in scarlet fever, which numerous investigators have persistently doubted, or perhaps sulfanilamide has no effect on the toxins of hemolytic streptococci which may be operating from a focus safe from the effects of the drug.

Acute Rheumatic Fever.—Rheumatic fever is another disease often believed to be associated with hemolytic streptococci in which sulfanilamide has no beneficial effects. In careful studies Swift, Moen and Hirst [44] were unable to shed much new light on the subject except

40. Stafford, J.: Prontosil, Science (supp.) **85**:9-10 (June 18) 1937.

41. Snodgrass, W. R., and Anderson, T.: Prontosil in Treatment of Erysipelas: Controlled Series of Three Hundred and Twelve Cases, Brit. M. J. **2**:101-104 (July 17) 1937. Breen, G. E., and Taylor, I.: Erysipelas Treated with Prontosil, Lancet **1**:1334-1335 (June 5) 1937.

42. Colebrook, L., and Purdie, A. W.: Treatment of One Hundred and Six Cases of Puerperal Fever by Sulphanilamide (Streptocide), Lancet **2**:1291-1293 (Dec. 4) 1937.

43. Hogarth, J. C.: Para-Benzylaminobenzenesulphonamide in the Treatment of Scarlet Fever, Brit. M. J. **2**:1160-1162 (Dec. 11) 1937.

44. Swift, H. F.; Moen, J. K., and Hirst, G. K.: The Action of Sulfanilamide in Rheumatic Fever, J. A. M. A. **110**:426-434 (Feb. 5) 1938.

to discuss the possible effects of the drug on streptococci which may be harbored in the tonsils or elsewhere to serve as hypothetic foci of infection. The administration of sulfanilamide apparently has no effect on hemolytic streptococci located in the tonsils or in the lochia of women who recover from puerperal infection. Massell and Jones [44a] also report that they have found sulfanilamide to be ineffctive in influencing the course of rheumatic fever. Sulfanilamide [45] was without effect in controlling experimental infection with staphylococci. At the meeting of the Association of American Physicians held in May 1938 Bauer reported no success in the treatment of rheumatoid arthritis with sulfanilamide.

Pneumococcic Infections.—Infection experimentally induced in animals with pneumococci of types I,[46] II, III [47] and XIV [48] were effectively controlled by the administration of sulfanilamide. In many of these experiments the amount of the drug that had to be given to obtain a satisfactory cure was far in excess of the amount tolerated by human beings in proportion to body weight.

Gonococcic Infections.—Most reports concerning the treatment of gonorrhea with sulfanilamide have been favorable, yet the studies have often been uncontrolled, and in many cases the patients treated were not observed long enough after the supposed cure. It is still too early to judge the value of the drug for this infection. The tone of one paper, that of Johnson and Pepper,[49] is pessimistic. More than half the patients treated were not sufficiently benefited so that curative power could be ascribed to the drug. The authors do not recommend therapy with sulfanilamide as a type of treatment to be employed for ambulatory patients.

44a. Massell, B. F., and Jones, T. D.: Effect of Sulfanilamide on Rheumatic Fever and Chorea, New England J. Med. **218**:876-877 (May 26) 1938.

45. Mellon, R. R.; Shinn, L. E., and McBroom, J.: Therapy of Experimental Staphylococcus Infections with Sulfonamide Compounds, Proc. Soc. Exper. Biol. & Med. **37**:563-565 (Dec.) 1937.

46. Kreidler, W. A.: Treatment of Pneumococcal Infections in Rabbits with Sulfanilamide, Proc. Soc. Exper. Biol. & Med. **37**:146-149 (Oct.) 1937.

47. Cooper, F. B., and Gross, P.: Para-Aminobenzenesulfonamide Therapy in Experimental Type III Pneumococcal Pneumonia, Proc. Soc. Exper. Biol. & Med. **36**:678-681 (June) 1937; Sulfanilamide, Antipneumococcus Serum and Vitamin C Therapy in Type II Pneumococcal Pneumonia of Rats, ibid. **36**:774-776 (June) 1937.

48. Schmidt, L. H.: Use of Sulfanilamide in the Treatment of Type XIV Pneumococcus Infections in Mice, Proc. Soc. Exper. Biol. & Med. **37**:205-206 (Oct.) 1937.

49. Johnson, H. S., and Pepper, S. D.: The Evaluation and Danger of the Treatment of Gonorrhea with Derivatives of the Sulfonamide-Azo Dyes, Week. Roster **33**:465 (Dec. 11) 1937.

Other Infections.—Both experimental and clinical studies [35] indicate a favorable effect of sulfanilamide on infections caused by the meningococcus. A combination of immune serum and drug therapy seems most effective.[50] Two English physicians [51] report success in the treatment of undulant fever with sulfanilamide. In the 4 patients treated recovery occurred after the drug had been given. The effects are not wholly convincing. More striking results are reported by Stern and Blake.[52]

Rich and Follis [53] found sulfanilamide to have striking effects in inhibiting the development of tuberculosis in guinea pigs experimentally infected with tubercle bacilli. When the animals were given small doses (100 mg. per day) the effects were not good, but when doses of 500 mg. were given daily the tuberculous process was well controlled. Under somewhat different experimental conditions Smithburn [53a] was unable to detect any influence of sulfanilamide in guinea pigs inoculated with tubercle bacilli.

Both sulfanilamide and mandelic acid appear to be of value in the treatment of infections of the urinary tract, especially if colon bacilli or hemolytic streptococci are operative.[54] Sulfanilamide exerts its best effects when the urine is acid, preferably at a p_H of 5.5 or less. The drug has no value if infection is caused by group D hemolytic streptococci.

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50. Branham, S. E., and Rosenthal, S. M.: Studies in Chemotherapy: V. Sulfanilamide, Serum and Combined Drug and Serum Therapy in Experimental Meningococcic and Pneumococcic Infections in Mice, Pub. Health Rep. **52**:685-695 (May 28) 1937.

51. Richardson, L. A.: Infection with Brucella Abortus Treated with Prontosil, Lancet **1**:495-496 (Feb. 26) 1938. Francis, A. E.: Sulfanilamide in the Treatment of Undulant Fever, ibid. **1**:496-497 (Feb. 26) 1938.

52. Stern, R. L., and Blake, K. W.: Undulant Fever: Its Treatment with Sulfanilamide, J. A. M. A. **110**:1550-1551 (May 7) 1938.

53. Rich, A. R., and Follis, R. H.: Inhibiting Effect of Sulfanilamide on the Development of Experimental Tuberculosis in the Guinea Pig, Bull. Johns Hopkins Hosp. **62**:77-84 (Jan.) 1938.

53a. Smithburn, K. C.: Inefficacy of Prontylin in Experimental Tuberculosis, Proc. Soc. Exper. Biol. & Med. **38**:574-575 (May) 1938.

54. Kenny, M.; Johnston, F. D., and Von Haebler, T.: *p*-Aminobenzenesulfonamide in Treatment of Bacterium Coli Infections of the Urinary Tract, Lancet **2**:119-125 (July 17) 1937. Walther, H. W. E.: Urinary Antisepsis, J. A. M. A. **109**:999-1004 (Sept. 25) 1937. Helmholz, H. F.: A Comparison of Mandelic Acid and Sulfanilamide as Urinary Antiseptics, ibid. **109**:1039-1041 (Sept. 25) 1937. Bliss, E. A., and Long, P. H.: Failure of Para-Aminobenzenesulfonamide Therapy in Urinary Tract Infections Due to Group D (Lancefield) Beta Hemolytic Streptococci, New England J. Med. **217**:18-21 (July 1) 1937.

Several studies [55] suggest the effectiveness of sulfanilamide in the treatment of malaria, but Hill and Goodwin,[56] who tried it on 100 patients and obtained good results, do not recommend its use, because of its toxic effects. The drug had no therapeutic value in the treatment of mice inoculated with Bacillus pertussis [57] but was apparently effective in the treatment of "sniffles" [58] of rats kept in laboratory colonies. A few patients with bubonic plague recovered after receiving prontosil, a derivative of sulfanilamide (the disodium salt of 4-sulfamidophenyl-2′-azo-7′-acetylamino-1′-hydroxynaphthalene-3′,6′-disulfonic acid).[59]

Investigators [60] at the National Institute of Health found prontosil [61] to have a protective action in mice against infection with the virus of lymphocytic choriomeningitis when large doses were given shortly after inoculation with small amounts of virus. The drug or its related compounds had no effect on infections caused by the viruses of St. Louis encephalitis or influenza. Others [61a] have obtained more promising results in the treatment of experimental influenza. Dochez and Slanetz [62] obtained results which indicated the effectiveness of sodium sulfanilylsulfanilate ($NH_2.C_6H_4.SO_2.NH.C_6H_4.SO_3Na$) when given to ferrets inoculated with the virus of canine distemper. The drug prevented the disease promptly when administered shortly after the development of symptoms and fever. Secondary bacterial infection seemed at times to hamper the favorable action of the drug. Marcus and Necheles [63] report good results in treating spontaneous infection of distemper in dogs with

55. van der Wielen, Y.: Prontosil in Quartan Malaria, Nederl. tijdschr. v. geneesk. **81**:2905-2910 (June 19) 1937. Diaz de Leon, A.: Treatment of Malaria with Sulfonamide Compounds, Pub. Health Rep. **52**:1460-1462 (Oct. 15) 1937.

56. Hill, R. A., and Goodwin, M. H.: Prontosil in the Treatment of Malaria, South. M. J. **30**:1170-1171 (Dec.) 1937.

57. Gross, P.; Cooper, F. B., and Lewis, M.: Chemotherapy of B. Pertussis Infections of Mice, Proc. Soc. Exper. Biol. & Med. **38**:407 (April) 1938.

58. Maier, N. R. F.: The Treatment of "Sniffles" in the Rat with Sulfanilamide, Science **87**:439 (May 13) 1938.

59. Carman, J. A.: Prontosil in the Treatment of Oriental Plague, East African M. J. **14**:362-365 (Feb.) 1938.

60. Rosenthal, S. M.; Wooley, J. G., and Bauer, H.: Studies in Chemotherapy: VI. The Chemotherapy of Choriomeningitis Virus Infection in Mice with Sulphonamide Compounds, Pub. Health Rep. **52**:1211-1217 (Sept. 3) 1937.

61. The prontosil used was 4-sulfamido-2′-4′-diaminoazobenzene hydrochloride.

61a. Climenko, D. R.; Crossley, M. L., and Northey, E. H.: The Protective Action of Certain Sulfanilamide Derivatives in Experimental Influenza Infections, J. A. M. A. **110**:2099-2100 (June 18) 1938.

62. Dochez, A. R., and Slanetz, C. A.: The Treatment of Canine Distemper with a Chemotherapeutic Agent, Sodium Sulfanilyl Sulfanilate, Science **87**:142-144 (Feb. 11) 1938.

63. Marcus, P. M., and Necheles, H.: Treatment of Spontaneous Canine Distemper with Sulfanilamide, Proc. Soc. Exper. Biol. & Med. **38**:385-387 (April) 1938.

the drug, but the experience of Dickerson and Whitney [64] was disappointing in this regard. No evidence was found for the effectiveness of the drug in the disease.

The foregoing brief review clearly indicates that sulfanilamide and its related compounds are remarkable chemotherapeutic agents with unlimited possibilities for future development. But it is unwise perhaps to expect too much. One has but to recall the enthusiasm with which arsphenamine was received as a possible cure for all infections, yet in spite of years of effort, no modifications of it have been found to influence the course of any infection aside from a few which are caused by spirochetes. It is far too early to estimate the actual value of sulfanilamide in many of the infections mentioned. In most reports of experimental studies attention has been directed to the necessity of using large amounts of the drug and small doses of infective agent. Many more cases will have to be carefully studied together with controls before it will be wise to use the drug in general practice except for the few infections against which it is known to be of value. Despite the warnings which have been repeatedly given, the indiscriminate use of the drug for any febrile disease is deplorable. The toxic effects of the drug will not be considered here. Suffice it to say that cyanosis, jaundice, agranulocytosis, anemia, optic neuritis, cutaneous eruptions, fever and other unpleasant and dangerous side effects have been reported, many of them in the Sept. 25, 1937, issue of *The Journal of the American Medical Association.*

Many investigators are at present attempting to discover new compounds which have a greater toxic effect on the infecting agents and are less toxic for the host. Bauer and Rosenthal [65] report that disulfanilamide (paraaminobenzenesulfonylsulfonamide, $NH_2.C_6H_4.SO_2NH.C_6H_4.SO_2NH_2$) administered orally had a therapeutic index of 5, while that of sulfanilamide was only 3.3. Another compound, studied by Buttle, diaminodiphenylsulfone ($NH_2.C_6H_4.SO_2.C_6H_4.NH_2$), was thirty times as active against streptococci as sulfanilamide but was also highly toxic for the host. Fourneau used an acetyl derivative of diaminodiphenylsulfone ($CH_3.CO.HN.C_6H_4.SO_2.C_6H_4.NH.COCH_3$), and Bauer used a water-soluble formaldehyde sulfoxylate derivative ($NaSO_2.CH_2.HN.C_6H_4.SO_2.C_6H_4.NH.CH_2.SO_2Na$). All three of these sulfones are claimed to be superior to sulfanilamide for treatment of pneumococcic infections in mice, but, as Bauer and Rosenthal conclude, "in mice the action is still considerably less marked than against

64. Dickerson, V. C., and Whitney, L. F.: Sulfanilamide and Prontosil in the Treatment of Canine Distemper, Proc. Soc. Exper. Biol. & Med. **38**:263-264 (March) 1938.

65. Bauer, H., and Rosenthal, S. M.: Studies in Chemotherapy: VII. Some New Sulphur Compounds Active Against Bacterial Infections, Pub. Health Rep. **53**:40-49 (Jan. 14) 1938.

streptococci; and, while marked prolongation of life can be achieved, few animals permanently survive pneumococcal infections as a result of therapy." Other workers [66] have found 4,4′-diacetylaminodiphenylsulfone to be more effective than sulfanilamide in hemolytic streptococcic infection of mice. It is also less toxic for mice.

ACUTE RHEUMATIC FEVER AND RHEUMATOID ARTHRITIS

British investigators [67] present further evidence to establish an etiologic relation between certain "elementary bodies" and acute rheumatic fever. The elementary bodies are found in the serous effusions in acute rheumatic fever and in other diseases as well, but the ones found in rheumatism are more uniform in size and more abundant. They are specifically agglutinated by serum from other patients with rheumatic fever. There is also some cross agglutination with serum from patients with rheumatoid (infectious) arthritis and chorea. This, the authors believe, supports the view that an etiologic relation between rheumatic fever, chorea and rheumatoid arthritis exists, as suggested by others.

Any one interested in the treatment of acute infections, particularly of acute rheumatic fever, will profit by reading a paper by Wilson [68] on the natural history of the disease. Much unwarranted enthusiasm arising from the supposedly curative effects of various remedies applied in treatment could be avoided if one realized how easy it is to be misled by the natural remissions of any disease. According to Wilson, the natural course of rheumatic fever as observed is apparently uninfluenced by any form of treatment. Jordan's [69] observations confirm these views as far as vaccine treatment for rheumatism is concerned. Vaccine therapy for rheumatic fever and rheumatoid arthritis, he believes, and I do also, rests on empiricism; no evidence exists to justify its use as being of specific value. Vaccines may be tried as an experimental procedure in selected cases if the patients are kept under close observation, but they should not be used routinely.

In another study Wilson and Schweitzer [70] endeavored to show the existence of a hereditary factor in the susceptibility to rheumatic fever.

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66. Cooper, F. B.; Gross, P., and Lewis, M.: Sulfone and Sulfanilamide Therapy in Streptococcal Infections, Proc. Soc. Exper. Biol. & Med. **38**:375-377 (April) 1938.

67. Eagles, G. H.; Evans, P. R.; Fisher, A. G. T., and Keith, J. D.: Virus Etiology of Rheumatic Disease, Lancet **2**:421-428 (Aug. 21) 1937.

68. Wilson, M. G.: The Natural History of Rheumatic Fever in the First Three Decades, J. Pediat. **10**:456-465 (April) 1937.

69. Jordan, E. P.: Critical Evaluation of Vaccine Therapy in Rheumatism, J. A. M. A. **109**:1444-1445 (Oct. 30) 1937.

70. Wilson, M. G., and Schweitzer, M. D.: Rheumatic Fever as a Familial Disease: Environment, Communicability and Heredity in Their Relation to Observed Familial Incidence of Disease, J. Clin. Investigation **16**:551-570 (July) 1937.

There appears to be a familial tendency for the disease to develop, but other factors, such as environment and exposure, no doubt are also essential for infection to take place. The authors conclude that susceptibility to rheumatic fever is transmitted as a single autosomal recessive gene. They observed that the resulting rheumatic activity had no direct relation to the type and the source of exposure. Rheumatic fever followed either active or inactive exposure. Intimate contact with a source of infection in a patient with active rheumatic fever, called familial exposure, was of no greater significance than casual contact or extrafamilial exposure in inciting the disease.

In a dietary study [71] of rheumatic fever, children with the disease were given a special diet of high caloric value and were compared with other children with rheumatic fever who received ordinary hospital food. There was a significant gain of weight in those who partook of the special diet, but no other beneficial effects were noted. Since gain in weight and good nutrition are usually desired in rheumatic fever, the special diet is recommended.

Bland and Jones [72] studied 306 fatal cases which occurred among 1,500 persons under the age of 21 with rheumatic fever. Rheumatic fever was the chief cause of death in 250 cases (82 per cent). The clinical manifestations in fatal cases of the disease differed somewhat from the typical picture; arthritis, for example, was not present in any case in the course of the terminal illness; abdominal and precordial pain were more troublesome. Chorea was rarely seen in these cases, subcutaneous nodules were present in 60 per cent but carditis was present in every case. Pericarditis was noted in about 35 per cent clinically and in 80 per cent post mortem. Evidence of pulmonary and hepatic involvement was common.

In further studies on the subject Gouley [73] points out the frequency with which pneumonitis occurs in patients with rheumatic fever, particularly with severe infection. The pulmonary inflammation in the earliest stages is not caused by secondary invading bacteria but is an integral part of the disease. The clinical manifestations are often overshadowed by other symptoms or overlooked if examination of the lungs is omitted. There is often marked disproportion between the mild symptoms referable to a pneumonic infiltration and the extent of the

71. Sadow, S. E.; Hubbard, J. P., and Jones, T. D.: A Dietary Study in Rheumatic Fever, New England J. Med. **217**:170-174 (July 29) 1937.

72. Bland, E. F., and Jones, T. D.: Fatal Rheumatic Fever, Arch. Int. Med. **61**:161-171 (Feb.) 1938.

73. Gouley, B. A.: The Acute and Subacute Pulmonary Involvement in Rheumatic Fever with Notes on the Complication of Basal Pulmonary Collapse, Ann. Int. Med. **11**:626-636 (Oct.) 1937.

lesion as shown by a roentgenogram. Respiratory distress is usually slight unless the consolidation is massive. The acute lesion may result in inflammatory collapse and subsequent fibrosis. The problem was also studied by Melnick.[73a]

Only a few of the papers dealing with rheumatic diseases are mentioned here. For a comprehensive review of the subject the reader is referred to the "Fourth Rheumatism Review" of Hench and his associates.[74] This review of publications concerning one group of diseases alone, rheumatism, extracts information from over six hundred papers and books published in the English language in a single year and occupies 156 pages.

FOCAL INFECTION

Ash's [75] recent paper adds more data to the accumulating evidence with which the overenthusiasm in the concept of focal infection can be combated. She refers to Kaiser's valuable studies, which have done so much to check the indiscriminate removal of tonsils, and feels that even his conclusions are somewhat too optimistic in regard to the beneficial effects of tonsillectomy in rheumatic fever. Ash feels that it is debatable whether tonsils serve as the portal of entry or a focus of infection for the etiologic agent of rheumatic fever. Rheumatic fever occurs in persons whose tonsils have been removed and also in those in whom acute or chronic infection of the tonsils is absent. In the series of cases reported Ash found that tonsillectomy had no influence on the incidence of recurrent attacks; there was also no significant difference between the death rate for the children whose tonsils had been removed before the onset of the disease and that for the children whose tonsils were removed subsequently or had never been removed. Rheumatic exacerbations followed in a high proportion of cases in which the tonsils were extirpated early in the course of the disease. Similar findings had previously been reported by Wilson, Lingg and Croxford; Campbell and Warner, and Wallace and Smith. These conclusions can be reconciled with Kaiser's statistics, which showed that the mortality rate appeared to be much less for tonsillectomized children, by regrouping the statistics and analyzing them differently.

73a. Melnick, P. J.: Pulmonary Changes in Rheumatic Fever, Illinois M. J. **73**:336-339 (April) 1938.

74. Hench, P. S.; Bauer, W.; Ghrist, D.; Hall, F.; Holbrook, W. P.; Key, J. A., and Slocumb, C. H.: The Present Status of Rheumatism and Arthritis: Review of American and English Literature for 1936 (Fourth Rheumatism Review), Ann. Int. Med. **11**:1089-1247 (Jan.) 1938.

75. Ash, R.: Influence of Tonsillectomy on Rheumatic Infections, Am. J. Dis. Child. **55**:63-75 (Jan.) 1938.

No reconciliation is possible, however, with the unwarranted conclusions drawn by Turnley.[76] This author advises removal of tonsils in all cases of rheumatism, among which are included instances of "painful conditions of the muscles, fascia, bones, nerves and joints." His listing of conditions which he says are indications for tonsillectomy is more dangerous than ludicrous, for he mentions cheesy plugs in the tonsil, keratosis due to Leptothrix, conjunctivitis, optic neuritis, run-down conditions, harboring of diphtheria bacilli, cardionephritis, certain thyroid disorders and asthma. Worst of all, tonsillectomy is recommended as a prophylactic measure against tuberculosis and Hodgkin's disease. In a previous publication this author made the preposterous suggestion that there would be a healthier and better race if children had their tonsils and adenoids removed before the age of 5 or 6 years.

To complicate an obscure problem still further, Gehlen [77] regards polyarthritis as a spinal dystrophic articular disease resulting from the effects of a "toxin" elaborated in foci of infection. How the author arrives at his conclusions is a mystery, for no proof or bacteriologic studies of the existence of a toxin are presented. Gutzeit [78] states that diseased or infected teeth can be recognized by subjecting them to short wave irradiation with a specially adapted apparatus. When healthy teeth are irradiated, an acceleration of the sedimentation rate of the erythrocytes is not caused, but if "diseased" teeth are so treated the "toxic" substances present enter the circulation and disturb the suspension stability of the blood. He admits that the extraction of teeth giving a positive reaction did not always cause improvement in general symptoms.

Such papers as those of Turnley, Gehlen and Gutzeit are cited merely as examples to warn clinicians to scrutinize thoroughly all evidence before applying dangerous methods of treatment of any kind to their patients. Editors of medical journals could prevent the dissemination of much misinformation by demanding the inclusion of rigid control tests and by more liberal use of a blue pencil on unsupported expressions of opinion.

Cecil, once an advocate of the removal of foci of infection in the treatment of rheumatoid arthritis, has changed his mind on the subject.

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76. Turnley, W. H.: Tonsillectomy for Rheumatism: A Study of 3,172 Cases, Ann. Otol., Rhin. & Laryng. **46**:1050-1059 (Dec.) 1937; The Results of 76,000 Adenoid and Tonsil Operations, Laryngoscope **47**:1-6 (Jan.) 1937.

77. Gehlen, H.: Neuere Erkenntnisse auf dem Krankheitsgebiet der primär chronischen Polyarthritis und ihre Auswertung für die Therapie, Verhandl. d. deutsch. Gesellsch. f. inn. Med. **49**:377-383, 1937.

78. Gutzeit, K.: Zur Frage der dentalen Infektion und ihrer Diagnosestellung durch Kurzwellenprovokation der Zähne, München. med. Wchnschr. **85**:164-166 (Feb. 4) 1938.

In an address before the American College of Physicians last spring, he and Angevine gave the results of their follow-up study of patients with arthritis who had had various supposed foci of infection removed. In no instance was the course of the disease arrested, nor was the patient cured by tonsillectomy. Almost no benefit was obtained from the extraction of teeth or operations on the sinuses. They believe that the time has come for a revaluation of the theory of focal infection.

A study made in thirty-one schools in England [78a] led a committee to conclude: "Though realizing the value of operations in carefully selected cases, we have grave doubts as to whether the majority of tonsillectomies performed today are the result of true discrimination rather than of routine ritual." Similar views are expressed by Glover and others.[78b] In the United States tonsillectomy comprises one third of all surgical operations. Tonsillectomy was performed on at least three times as many persons who were well-to-do as on poorer ones. One can scarcely avoid the conviction that something must be gravely wrong in this practice and that it is time to call attention to the needlessness in most cases of removing the tonsils or teeth in the hope of preventing or influencing systemic diseases. I have attacked the concept of focal infections pertaining to teeth and tonsils in each of my three previous annual reviews in the ARCHIVES.

COCCIC DISEASES

Staphylococcic Infections.—Accumulating evidence is providing a more optimistic outlook for the treatment of staphylococcic infections. In reviews of the subject both Rigdon [79] and Bigger [80] write favorably of the therapeutic effects of staphylococcus antitoxins. According to Rigdon, vaccines have little therapeutic effect, but toxoid seems to be of value in certain cases of chronic infection. Antitoxin likewise appears to be of value in the treatment of certain acute staphylococcic infections accompanied by toxemia. Bigger strongly advises against surgical intervention during a staphylococcic infection except when liquefaction has occurred. Incision even then should never transgress the protective zone. He also favors the use of antitoxin and toxoid. Downie,[81] in

78a. Epidemics in Schools, Medical Research Council, Special Report Series, no. 227, London, His Majesty's Stationery Office, 1938.

78b. A Criticism of Tonsillectomy, Foreign Letters, J. A. M. A. **111**:181 (July 9) 1938.

79. Rigdon, R. H.: Staphylococcic Immunity: Résumé of Experimental and Clinical Studies, Arch. Path. **24**:233-245 (Aug.) 1937.

80. Bigger, J. W.: The Staphylococci Pathogenic for Man, Brit. M. J. **2**:837-841 (Oct. 30) 1937.

81. Downie, A. W.: A Comparison of the Value of Heat-Killed Vaccines and Toxoid as Immunizing Agents Against Experimental Staphylococcal Infection in the Rabbit, J. Path. & Bact. **44**:573-587 (May) 1937.

experimental studies, was unable to increase the resistance of rabbits to staphylococcic infections of the skin by vaccinating them with staphylococcus vaccines.

Neter [82] found that staphylococci from human sources may produce either fibrinolysin or anticoagulant, or they may possess both or neither of these properties. Staphylococci pathogenic for man may produce either fibrinolysin or anticoagulant. In patients infected with a staphylococcus able to cause fibrinolysis, a specific antifibrinolysin may develop. The fibrinolysin, unlike the one of the hemolytic streptococcus, dissolves clots of both human and animal blood. The anticoagulant likewise inhibits coagulation of human and of animal plasma. It can be neutralized by normal serum, and it is not antigenic. The plasma-clotting factor is sometimes present in exudates. It may be inhibited by the anticoagulant of staphylococci or of other bacteria without being destroyed.

Gonorrhea.—Vonderlehr and Usilton [83] made some important studies on the incidence of gonorrhea. At least a million new cases occur in the United States annually, and nearly half a million persons with the disease are under treatment; thus gonorrhea seems to be more prevalent than any other serious infectious disease. The incidence of the infection is greatest in small cities, and the mean age of persons acquiring the infection is about 24 years. There is no evidence that the incidence of gonorrhea is declining. Carpenter [84] made a comparative study of various methods for the diagnosis of gonorrhea. The culture method is the most reliable. The use of stained smears, although much simpler, may give rise to error, since other gram-negative cocci may be mistaken for gonococci. The complement fixation test as generally performed is not satisfactory or reliable. Warren and his associates [85] continue to report good results from fever therapy in gonorrheal infections. At the meeting of the Association of American Physicians in May, Bauer reported favorable results in the treatment of gonorrheal arthritis with sulfanilamide.

82. Neter, E.: Fibrinolytic, Anticoagulating and Plasma-Clotting Properties of Staphylococci, J. Bact. **34**:243-253 (Sept.) 1937.

83. Vonderlehr, R. A., and Usilton, L. J.: The Gonorrhea Problem in the United States, J. A. M. A. **109**:1425-1427 (Oct. 30) 1937.

84. Carpenter, C. M.: The Diagnosis of Gonococci Infection in the Male: An Evaluation of Laboratory Methods, J. A. M. A. **109**:1328-1430 (Oct. 30) 1937.

85. Warren, S. L.; Scott, W. W., and Carpenter, C. M.: Artificially Induced Fever for the Treatment of Gonococcic Infection in the Male, J. A. M. A. **109**:1430-1434 (Oct. 30) 1937.

The problem of latency of gonococcic infection was investigated by Spink and Keefer.[86] Persons may harbor gonococci in the body for years without symptoms to indicate their presence, yet when some unrelated circumstance, such as a cold or pregnancy occurs, the bacteria become active and cause disease. Gonorrheal arthritis may develop in a person who gives no history of previous urethritis.

Meningococcic Meningitis.—In classifying the types of meningococci obtained from patients with meningococcic infection since 1930, Branham and Carlin[87] found that among 409 strains, 86 per cent were of group I-III and only 11.7 per cent of type II. Among 42 strains isolated from healthy carriers, 57 per cent were of type II and 28 per cent of group I-III. Most of the persons from whom group I-III meningococci were recovered were in immediate contact with patients suffering from infection caused by the same types of meningococci. Type II meningococci were apparently relatively unimportant as a cause of disease in the period when the study was made.

In another study Branham and her associates[88] succeeded in causing purulent meningitis in guinea pigs by the intracisternal injection of living or heat-killed meningococci without the aid of mucin to enhance the virulence of the bacteria. Filtrates of broth cultures containing hypothetic toxins also caused the production of purulent exudate. Meningitis may therefore be caused both by infection and by intoxication. Antimeningococcus serum had no effect in protecting animals from meningitis.

A group of United States Army medical officers[89] took the opportunity of testing the value of intradermal reactions to discover persons liable to meningococcic infection and to study the effect of prophylactic immunization among members of the Civilian Conservation Corps. Fifty-three per cent of 7,339 subjects tested showed a 1 plus or greater reaction to the intradermal injection of meningococcus "toxin" or filtrate from broth cultures of meningococci. Those who reacted positively and were presumably susceptible to infection were given several injections of filtrate and were retested two months later. At this time 80

86. Spink, W. W., and Keefer, C. S.: Latent Gonorrhea as a Cause of Acute Polyarticular Arthritis, J. A. M. A. **109**:325-328 (July 31) 1937.

87. Branham, S. E., and Carlin, S. A.: A Study of Meningococci Recovered in the United States Since 1930, J. Bact. **34**:275-283 (Sept.) 1937.

88. Branham, S. E.; Lillie, R. D., and Pabst, A. M.: Experimental Meningitis in Guinea Pigs, Pub. Health Rep. **52**:1135-1142 (Aug. 20) 1937. Branham, S. E., and Pabst, A. M.: Serum Studies in Experimental Meningitis, ibid. **52**:1143-1150 (Aug. 20) 1937.

89. Kuhns, D. M.; Kisner, P.; Williams, M. P., and Moorman, P. L.: The Control of Meningococcic Meningitis Epidemics, J. A. M. A. **110**:484-487 (Feb. 12) 1938.

per cent showed a negative or nearly negative reaction. They report twenty outbreaks of meningitis in which it appeared that immunization with filtrates seemed to limit or prevent the spread of infection.

BACILLARY DISEASES

Tuberculosis.—Amberson [90] points out the importance of recognizing the early lesion of pulmonary tuberculosis, which, in general, the members of the medical profession seem rather slow to comprehend. Most often in early life the first effect of infection with tubercle bacilli is the development of a small patch of pneumonia usually in the upper portion of one lung. The symptoms arising from the lesion are usually so mild as to be unrecognized or to be regarded as the result of an unimportant infection of the respiratory tract. Abnormal physical signs are usually absent. As a matter of fact, in most cases the pneumonic area is discovered accidentally in periodic routine health examinations or when roentgenograms of the chest are made after a tuberculin test is found to show a positive reaction. The early lesion may heal completely or may leave calcified remnants, but in unfavorable cases the area may caseate, spread and result in cavitation. The infection may remain dormant for many years before spreading. Similar views are expressed by Hetherington.[91] Myers does not approve of sending a patient with the first infection type of mild tuberculous pneumonia to a sanatorium. The patient should, he believes, be treated at home and should be kept in bed only until evidence of the lesion and the symptoms have disappeared.

Zavod [92] reviews several cases of hematogenous pulmonary tuberculosis probably caused by dissemination of tubercle bacilli by way of the pulmonary artery, which, in contradistinction to generalized miliary tuberculosis, is confined chiefly to the lungs. The prognosis is favorable in most cases.

Opie and Freund [93] tested the relative effects of the injection of BCG and heat-killed tubercle bacilli in producing immunity to infection with tubercle bacilli. Heat-killed bacilli gave rise to immunity only slightly less in effectiveness than that produced by BCG. The injection of BCG frequently caused suppuration and abscess formation which healed spontaneously. Cutaneous sensitivity to tuberculin disappeared

90. Amberson, J. B.: The Lasting Cure of Early Pulmonary Tuberculosis, J. A. M. A. **109**:1949-1952 (Dec. 11) 1937.

91. Hetherington, H. W.: The Diagnosis and Management of Latent, Suspected and Early Clinical Tuberculosis, J. A. M. A. **109**:1952-1955 (Dec. 11) 1937.

92. Zavod, W. A.: Hematogenous Pulmonary Tuberculosis, J. A. M. A. **109**: 1693-1698 (Nov. 20) 1937.

93. Opie, E. L., and Freund, G.: An Experimental Study of Protective Inoculation with Heat Killed Tubercle Bacilli, J. Exper. Med. **66**:761-788 (Dec.) 1937.

one or two years after injection of heat-killed bacilli. Other observers [94] injected BCG intradermally in children and produced cutaneous sensitivity in 80 per cent. Subcutaneous injection caused cutaneous sensitivity in 60 per cent of the cases. Sensitivity reached its maximum in six months and diminished rapidly, so that most children no longer gave positive reactions to tuberculin after two years.

Russian investigators [95] infected rabbits with tubercle bacilli and subsequently inoculated them with pneumococci. The tuberculous rabbits were able to cope with pneumococci inoculated intravenously or intraperitoneally as well as healthy control rabbits, but in the former the reaction took a slower course, had a more exudative character and tended more to necrosis.

Aronson and Meranze [96] studied the effect of syphilis on experimentally produced tuberculous lesions in rabbits. In syphilitic rabbits the local inflammatory reaction after the injection of tubercle bacilli into the skin was more prompt and intense than after similar injections in normal rabbits. Histologically the lesions in the syphilitic animals resembled the lesions characteristic of an initial syphilitic infection, suggesting that the cells of the rabbit are so modified by the disease that the introduction of an unrelated bacterium elicits an inflammatory reaction characteristic of the initial syphilitic lesion. Later, the appearance of epithelioid cells and caseation resembled the usual reaction to tubercle bacilli.

In a scholarly paper Long [97] discusses the great variability of diseases caused by different varieties of mycobacteria, including tuberculosis, leprosy, "skin-lesion" disease of cattle, Johne's disease and rat "leprosy." These diseases are generally characterized by the presence of bacilli which are regarded as acid fast because of the presence of certain related chemical substances. The tissue responses to infection are similar in these diseases; there are extensive proliferation and accumulations of monocytes, which develop into "epithelioid" cells.

In a critical review Miller [97a] discusses a number of unsolved problems relating to tuberculosis which deserve thoughtful consideration.

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94. Kereszturi, C.; Rosenberg, H. A., and Park, W. H.: Tuberculin Allergy Produced by Parenteral BCG Vaccination, Am. Rev. Tuberc. **36**:90-99 (July) 1937.

95. Weissfeiler, J.; Morozova, E. N., and Strukov, G. I.: The Course of Experimental Pneumococcus Infection in Tuberculous Rabbits, Am. Rev. Tuberc. **37**:93-99 (Jan.) 1938.

96. Aronson, J. D., and Meranze, D. R.: The Effect of Syphilis on Local Tuberculous Lesions in Rabbits, Am. J. Path. **14**:163-175 (March) 1938.

97. Long, E. R.: Tuberculosis, Leprosy and Allied Mycobacterial Diseases, Science **87**:23-31 (Jan. 14) 1938.

97a. Miller, J. A.: Some Unsolved Problems of Tuberculosis, J. A. M. A. **111**:111-117 (July 9) 1938.

Undulant Fever.—An excellent description [98] of the pathologic anatomy of undulant fever has been published in Germany. Drei [99] calls attention to hepatomegaly as a sequela of undulant fever. Another investigator [100] in Italy showed that flies can be experimentally infected with Brucella and may serve as carriers for over a week. Deem [101] recovered Brucella abortus from 9 of 15 horses with fistulous withers or poll evil. The blood of 82 per cent of 34 horses with the disease agglutinated Brucella. Of 100 normal horses, 9.7 per cent gave positive reactions for Brucella in low dilution.

In a clinical study Beatty [102] points out the frequency with which pulmonary symptoms occur in undulant fever. Many patients, particularly those with the chronic type of infection, present themselves with symptoms of pulmonary involvement. They may complain of burning sensations or pain in the chest, malaise and cough without sputum or with mucoid, mucopurulent sputum. Hemoptysis is occasionally noted. The symptoms are likely to lead to an erroneous diagnosis of pulmonary tuberculosis. Among 47 patients with undulant fever, 31 had symptoms of involvement of the respiratory tract, 18 had cough, 18 raised sputum, 5 had hemoptysis and 26 complained of pain in the chest. In several cases of acute involvement, small patches of dulness, with rales and bronchial breathing, were found. Roentgenograms showed infiltration near the hilus and an increase in density in the peribronchial areas.

Hardy, Frant and Kroll [103] point out how variable the incubation period of undulant fever is. Infection with the more virulent melitensis variety is followed by a comparatively short period of incubation, while in naturally infected persons small doses of the abortus variety may result in a prolonged period. Their study was made on 17 residents of New York who drank pasteurized milk during residence in the city but who were known to have drunk raw milk during a visit elsewhere. The incubation period of the disease in these patients was found to vary from one week to four months, with the average time much more

98. von Albertini, A., and Lieberherr, W.: Beiträge zur pathologischen Anatomie der Febris undulans Bang, Frankfurt. Ztschr. f. Path. **51**:69-97, 1937.

99. Drei, G.: Considerazioni cliniche sul reperto frequente di esatomegalia come postumo di febbre ondulante, Gior. di clin. med. **18**:1368-1377 (Oct. 30) 1937.

100. Negro, G.: Significato della mosca domestica nella diffusione della febbre ondulante, Gior. di batteriol. e immunol. **19**:17-29 (July) 1937.

101. Deem, A. W.: Brucella Abortus in Horses, J. Infect. Dis. **61**:21-25 (July-Aug.) 1937.

102. Beatty, O. A.: Manifestations of Undulant Fever in the Respiratory Tract, Am. Rev. Tuberc. **36**:283-289 (Aug.) 1937.

103. Hardy, A. V.; Frant, S., and Kroll, M. M.: The Incubation Period in Undulant Fever, Pub. Health Rep. **53**:796-803 (May 20) 1938.

prolonged than that of Br. melitensis infection as noted in Malta and tested experimentally in Puerto Rico.

Tularemia.—Two papers have been published which reemphasize the frequency with which tularemia affects the lungs.[104] In several patients, pulmonary symptoms were predominant and ushered in the disease. Three patients were sheepherders who were bitten by ticks prior to the onset of the disease, but it is uncertain as to whether the bacilli were inoculated in this manner or otherwise. All 4 patients recovered; 3 were treated symptomatically, and 1 was given antitularensis serum.

The frequency with which pneumonia is found in cases of tularemia brings out further resemblances between this disease and plague. Tularemia, like plague, occurs in three clinical forms: bubonic, pneumonic and septicemic or typhoid; the causative bacteria are closely related, similar animal species serve as reservoirs of infection and both diseases may be conveyed to man by direct contact with infected flesh or blood, by inhalation and by insects. A similar relation may pertain to the disease called pseudotuberculosis, as I pointed out several years ago.[105]

Foshay [106] claims that septicemia is the chief cause of death in cases of tularemia; the mortality rate of patients with tularemic pneumonia is about 30 per cent. It is true, of course, that the mortality rate is higher in most diseases when the blood stream is invaded, but whether the septicemia per se is chiefly responsible is not so certain. In persons severely ill with infection, bacteria, as a result, are able to survive in the blood stream. Foshay continues to report good results following the use of his antitularensis serum in the treatment of tularemia, but the product was not accepted by the Council on Pharmacy and Chemistry of the American Medical Association for inclusion in New and Nonofficial Remedies, the publication of other corroborative data being awaited.

An excellent review of the pathology of tularemia has been published by Lillie and his associates.[107]

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104. Winter, M. D.; Farrand, B. C., and Herman, H. J.: Tularemia, Pulmonic Form: Report of Four Recoveries, J. A. M. A. **109**:258-262 (July 24) 1937. Blackford, S. D., and Archer, V. W.: Roentgen Study of a Nonfatal Case of Bilateral Tularemic Pneumonia Treated with Specific Serum, ibid. **109**: 264-265 (July 24) 1937.

105. Reimann, H. A., and Rose, W. B.: The Similarity of Pseudotuberculosis and Tularemia, Arch. Path. **11**:584-588 (April) 1931.

106. Foshay, L.: Cause of Death in Tularemia, Arch. Int. Med. **60**:22-38 (July) 1937.

107. Lillie, R. D.; Francis, E., and Parker, R. R.: The Pathology of Tularemia, National Institute of Health Bulletin 167, United States Treasury Department, Public Health Service, 1937, pp. 1-217.

Tularemia has only recently been identified in Germany.[108] Characteristically it was thought to be a "new" disease, never having been reported before. Unnecessary alarm was immediately raised, and importation of wild rabbits from Czechoslovakia and Austria was prohibited. Tularemia was recognized for the first time in Czechoslovakia and Austria a year or two ago, but, as has been demonstrated in other countries, the disease has perhaps been enzootic in rodents as long as rodents have existed in the region. Cases in human beings in the past no doubt have been mistaken for cases of other diseases. Ticks from rabbits in Alaska have been found to be infected with Pasteurella tularensis.[108a]

Three patients with obscure illness resembling tularemia were studied in England.[109] All 3 had hunted rabbits in Ireland, and at about the same time an epizootic of undetermined origin among cats was reported. In 1 case operation was performed, and the liver was found to contain lesions like those observed in tularemia, yet agglutinins for Pasteurella tularensis were absent. Each patient had eosinophilia. The authors suggest that diseases resembling tularemia and caused by closely related bacteria exist. Their suggestion brings to mind the possibility of the occurrence of a disease in man caused by Pasteurella pseudotuberculosis, a bacterium occasionally found in diseases of animals. Three French authors [110] report a case of pseudotuberculosis which resembled typhoid with icterus. A rat infected with P. pseudotuberculosis was trapped in Washington, D. C.[110a] The lesions produced by inoculating the bacilli into other animals resembled those of plague.

Plague.—Studies by Eskey [111] show that not all varieties of fleas are important in the transmission of plague. Female fleas seemed to be more dangerous as transmitters than males. In warmer localities where Xenopsylla cheopis is the only type of rat flea found, epizootics flare up and die out quickly, for the flea soon succumbs to infections. The flea readily transmits plague to man. In cold climates the Cera-

108. Tularemia in Germany, Foreign Letters, J. A. M. A. **110**:1502-1503 (April 30) 1938.

108a. Philip, C. B., and Parker, R. R.: Occurrence of Tularemia in the Rabbit Tick (Haemaphysalis Leporis-Palustris) in Alaska, Pub. Health Rep. **53**:574-575 (April 15) 1938.

109. Thomson, A. P.; Wilson, G. H., and McDonald, S.: Three Cases of Unusual Illness with Eosinophilia, One with Lesions in the Liver Resembling Those of Tularemia, Lancet **2**:9-11 (July 3) 1937.

110. Dujardin-Beaumetz, E.; Ballet, B., and Cebron, J.: Pseudo-tuberculose chez l'homme, Presse méd. **46**:43-44 (Jan. 8) 1938.

110a. Haas, V. H.: A Study of Pseudotuberculosis Rodentium Recovered from a Rat, Pub. Health Rep. **53**:1033-1038 (June 24) 1938.

111. Eskey, C. R.: Recent Development in Our Knowledge of Plague Transmission, Pub. Health Rep. **53**:49-57 (Jan. 14) 1938.

tophyllus fasciatus variety is much less dangerous to man. The unusually low incidence of plague in human beings transmitted from wild rodents in the western part of the United States (sylvatic plague) is believed to be due to the fact that the fleas of these animals do not attack man. Only 40 cases in human beings have been recorded, and in many of these cases infection was probably acquired by direct contact with infected tissue or blood without the agency of fleas, and in others evidence shows that infection may have occurred from bites of domestic rat fleas (murine plague). The transmission of plague to man therefore seems to depend largely on the species of flea involved. Meyer [112] in California reports several cases of plague in human beings contracted in much the same way as is tularemia, that is, apparently directly from a sick cat and a rabbit, from a dead chipmunk and by the bite of a squirrel. All 4 patients recovered.

Meyer and his associates [113] report an interesting case of chronic relapsing latent meningeal plague in a boy of 10 in whom a bubonic form of plague developed in June 1934. The child recovered to some extent but became severely ill again in July with meningeal symptoms. Improvement again occurred, until a fatal relapse followed in October. Necropsy revealed meningitis caused by Pasteurella pestis. Meyer reviews the literature and finds numerous examples of plague meningitis and of cases in which plague bacilli were recovered from patients many months after their original illness. He remarks that for twenty years many persons have studied the form of plague (sylvatic) which occurs in California, yet none has contracted the disease. It is apparently a different and less contagious form than murine or rat-borne plague. Meyer's study further emphasizes the clinical similarities between plague and tularemia.

Typhoid.—Topley [114] reviews the facts pertaining to the effectiveness of antityphoid vaccination and points out several important features: (*a*) Antityphoid vaccination, though it reduces the risk from occasional contact infection, may be much less effective when vaccinated persons are submitted to severe or continuous infection. (*b*) Endemic or epidemic typhoid cannot be controlled by prophylactic vaccination alone. (*c*) Typhoid when it occurs in persons previously vaccinated may be as severe as that in unprotected persons, and the blood stream is often invaded. (*d*) Antityphoid vaccination properly conducted reduces the

112. Meyer, K. F.: The Sylvatic Plague Committee, Am. J. Pub. Health **27**:777-785 (Aug.) 1937.

113. Meyer, K. F.; Connor, C. L.; Smyth, F. S., and Eddie, B.: Chronic Relapsing Latent Meningeal Plague, Arch. Int. Med. **59**:967-980 (June) 1937.

114. Topley, W. W. C.: The Role of Active or Passive Immunization in the Control of Enteric Infection, Lancet **1**:181-186 (Jan. 22) 1938.

morbidity rate in general from four to six times. (*e*) There is an impression that persons with latent typhoid infection or during the incubation period of the disease should not be vaccinated lest "provocative typhoid" result. Provocative typhoid is presumably associated with a sudden onset, a severe course and a mortality rate much higher (44 per cent) than that for the usual forms. (No instructions are given how to determine whether or not a person is about to become ill with typhoid.) (*f*) Vaccination with typhoid vaccine is a preventive measure of major importance when persons living in an uninfected area with well developed sanitary services migrate to a place where enteric infection is endemic. (*g*) Adequate bacteriologic control of water, milk and food and control of carriers throughout the country would, with no inconvenience to the general public, reduce the incidence of enteric fever to a point at which a discussion of the role of antityphoid vaccination would bcome of academic interest, at least to those who stay at home.

Tetanus.—Abel's associates performed further experiments to illustrate the dual action of tetanus toxin. In previous studies two distinct and separate actions of tetanus toxin were shown: One is represented by the response of the central motor neurons, which is manifested by the hyperactive reflex symptoms; and the other is represented by the direct peripheral action of the toxin on the voluntary muscles or their motor end plates. The latter effect is responsible for the long-continued rigidity of the muscles and is clearly evident in a condition which has long been called local tetanus. Under these circumstances there may be rigidity and contracture of one or more extremities without any signs of tactile reflex motor tetanus. These effects never cause death directly unless muscles of vital function are involved, such as those of the pharynx, larynx or diaphragm.

Firor and Jonas [115] injected minute amounts of tetanus toxin directly into the anterior horns of the spinal cord. The only observable symptoms resulting therefrom were reflex hyperexcitability and reflex jerks or spasms brought about by the slightest tactile stimulus. In the quiet intervals between the reflex attacks the muscles were relaxed and flaccid. The cause of muscle rigidity is therefore to be sought not in the action of the toxin on special centers but in its action on the voluntary muscles. The experiments further show the improbability of an upward passage or diffusion of tetanus toxin within the cord and favor the hypothesis of the blood-borne distribution of tetanus toxin.

The results of Abel's work in the past few years have revealed the futility of the use of massive doses of tetanus antitoxin in the treatment of tetanus. Yet his papers have been apparently overlooked by many.

115. Firor, W. M., and Jonas, A. F.: Researches on Tetanus: IV. The Production of Reflex Motor Tetanus by Intraspinal Injection of Tetanus Toxin, Bull. Johns Hopkins Hosp. **62**:91-109 (Feb.) 1938.

One has but to read the report of a case by Cables [116] to realize the extent to which uncontrolled enthusiasm can influence therapy. In this case, a man with obviously mild tetanus was given 3,460,000 units of tetanus antitoxin over a period of forty-one days; the last dose was injected two days before the patient walked home. The cost of the antitoxin used, according to current prices, may be estimated at $1,600. The therapeutic procedure used in this case might be regarded more as a test of endurance or capacity than as having any clinical or scientific value.

Gilles [117] made a systemic investigation of the presence of tetanus bacilli or spores in the dust of the streets of a large city. He found Clostridium tetani to be widely distributed in street dust in spite of the minimal amounts of horse manure present in modern times. The results of his study justify the use of prophylaxis against tetanus in all cases of street accidents accompanied by lacerations or abrasions of the skin.

Tetanus toxoid: A year after the injection of three doses of alum toxoid [118] the antitoxic titer of the blood dropped considerably but remained fairly constant when three doses (0.5, 1 and 1 cc.) of the untreated toxoid were injected. A subsequent dose of either preparation given one year after the initial injections increased the antitoxic titer of the blood from twenty to fifty times. This level of protection is higher and more persistent than that resulting from the usual prophylactic effect of the usual form of tetanus antitoxin.

Leprosy.—Two members of the United States Public Health Service dispel a certain amount of confidence which has gradually accumulated concerning knowledge of leprosy. At the International Leprosy Conference in Cairo, Egypt, Hasseltine stated that there is no specific remedy for the disease. Apparently he and many others have but little faith in the power of chaulmoogra oil or its derivatives to influence leprosy. Wade and others, however, still regard it as a valuable drug. Hasseltine outlined a plan for the establishment of a national preventorium to which children of leprous parents may be admitted immediately after birth, cared for and educated at government expense. Although the mode of infection of leprosy is unknown, there is reason to believe that the disease, like tuberculosis, is acquired not by inheritance but by long exposure to a source of infection.

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116. Cables, H. A.: The Amount of Antitoxin Used in a Case of Tetanus, J. A. M. A. **110**:1271-1272 (April 16) 1938.

117. Gilles, E. C.: The Isolation of Tetanus Bacilli from Street Dust: Its Bearing on Surgical Practice, J. A. M. A. **109**:484-486 (Aug. 14) 1937.

118. Jones, F. G., and Moss, J. M.: Studies on Tetanus Toxoid: Response of Human Subjects to an Injection of Tetanus Toxoid or Tetanus Alum Precipitated Toxoid One Year After Immunization, J. Immunol. **33**:183-190 (Sept.) 1937.

McCoy [119] reiterates that the exact mode of transmission of the disease is unknown and points pessimistically to the fact that there exists no real proof of the efficiency of segregation as a measure for public health. Segregation is justified, he believes, from the point of view of the esthetic interests of a community and from that of public charity. Segregation, he believes, should be leniently enforced, particularly in regions where leprosy has no tendency to spread. Although prolonged and intimate contact seems necessary for infection to take place, nevertheless there are many cases in which no contact with known leprosy can be discovered. With the latter type of case one can only speculate as to the occurrence of latent leprosy or possible carriers as sources of infection. No laboratory animals have yet been found to be susceptible to infection with leprosy.

VIRUS DISEASES

Acute Anterior Poliomyelitis.—The question of the portal of entry of the virus of poliomyelitis has again assumed importance because of interest in the use of nasal sprays in attempted prophylaxis. A number of studies have cast doubt on the efficacy of trinitrophenol-alum sprays in preventing the disease. Numbers of "protected" persons have contracted poliomyelitis. The following statement [120] was recently endorsed by several leading authorities:

> There is at present no generally accepted preventive of poliomyelitis nor any effective remedy in the acute stage, other than absolute rest. Complete rest is so important in the early days of this inflammation of the central nervous system that it is usually far better to leave the child in bed at home when the disease is first suspected than to move him any appreciable distance to a hospital.

A recent polemic between Harmon [121] and Lennette [122] brings out a number of points in favor of routes of infection other than the nasopharynx. Harmon favors the gastrointestinal route of infection, as emphasized for years by Swedish investigators and by Toomey, since (1) there is no obvious contagion in poliomyelitis of man; (2) cutting the olfactory tracts does not prevent infection when the virus is inoculated intravenously; (3) monkeys do not contract the disease by the nasopharyngeal route unless heroic methods are used; (4) nasal spraying with zinc sulfate does not prevent infection when virus is given intra-

119. McCoy, G. W.: Communicability of Leprosy and Application of Control Measures, Arch. Dermat. & Syph. **37**:169-174 (Feb.) 1938.

120. Stimson, P. M.: Statement About Infantile Paralysis, J. A. M. A. **110**: 1620 (May 7) 1938.

121. Harmon, P. H.: The Use of Chemicals as Nasal Sprays in the Prophylaxis of Poliomyelitis in Man, J. A. M. A. **109**:1061 (Sept. 25) 1937.

122. Lennette, E. H.: The Use of Chemicals as Nasal Sprays in the Prophylaxis of Poliomyelitis in Man, J. A. M. A. **109**:1381-1382 (Oct. 23) 1937.

venously or by mouth; (5) symptoms in some cases are present exclusively in the gastrointestinal tract; (6) the disease can be produced experimentally in monkeys by gastrointestinal inoculation; (7) the virus may be found in the nasopharynx after intracerebral or intravenous inoculation; (8) it is often impossible to isolate the virus from the nasopharynx of patients, and it can often be recovered from the rectal washings; (9) alterations in the olfactory bulbs are not always present in patients examined post mortem.

Lennette in defending the concept of the nasopharynx as a portal of entry replies that (1) the presence of virus in the stool and rectal washings may be explained by virus which has been swallowed; (2) virus may not be recoverable from the nasopharynx of patients because of its presence in subinfective dosage or simultaneous presence with a neutralizing antibody; (3) virus has been recovered from the nasopharynx of a significant percentage of patients and contact carriers, and (4) his own experiments strongly suggested that infection does not occur in test animals if the olfactory nerves are cut. Lesions occur in the olfactory bulbs of animals inoculated intravenously, but complete serial sections must often be made to detect them. Each observer is critical of the size of the inoculum used by the other, claiming that overdosage may lead to erroneous conclusions.

The performance of some decisive experiments to show whether one route or the other or both may be followed by the virus in carriers of infection has not as yet been accomplished. The controversy may perhaps be clarified in part by the observation of Trask and Paul [123] that a generalization as to the route of infection does not apply to all strains of the virus. With some strains, for example, it is easy to infect monkeys by intracutaneous inoculation. Cutaneous infectivity seems to decrease with the age of the strain. Howe and Ecke [124] excised the olfactory tracts of monkeys and left the olfactory bulbs. In such animals after inoculation, fever developed but no paralysis. Other monkeys from which the olfactory bulbs were removed did not have fever or paralysis. The experiments are believed to suggest that the olfactory bulbs are the seat of the virus reaction. Schultz and Raffel [125] were able to concentrate the virus of poliomyelitis by means of ultracentrifugation in a vacuum.

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123. Trask, J. D., and Paul, J. R.: The Skin Infectivity of Poliomyelitis Virus, Science **87**:44-45 (Jan. 14) 1938.

124. Howe, H. A., and Ecke, R. S.: Experimental Poliomyelitis Without Paralysis, Proc. Soc. Exper. Biol. & Med. **37**:125-126 (Oct.) 1937.

125. Schultz, E. W., and Raffel, S.: Sedimentation of Poliomyelitis Virus by Means of a Vacuum Ultracentrifuge, Proc. Soc. Exper. Biol. & Med. **37**:297-299 (Nov.) 1937.

In an epidemic of poliomyelitis in Texas, Gilliam and Decherd [126] noted a large proportion of patients with predominant signs of bulbopontile involvement. Hudson and Lennette [127] obtained samples of serums from persons in Brazil and from an island near Australia to determine whether or not a specific virus-neutralizing substance was present to indicate that poliomyelitis occurs in these tropical areas. The serum from the majority of persons tested contained neutralizing properties, showing that poliomyelitis is probably worldwide in its distribution.

Encephalitis.—Investigators [128] in St. Louis isolated the virus which caused the 1937 outbreak of encephalitis and found it to be immunologically identical with the one which caused an epidemic in that city in 1933. An endemic focus no doubt exists there. Evidence [129] indicates that encephalitis of the St. Louis type was present in an epidemic in California in the summer of 1937. Fifty-five per cent of 29 samples of serum from patients with acute encephalitis or polioencephalitis neutralized the virus of the St. Louis type, and none neutralized virus of lymphocytic choriomeningitis. Other interesting studies [130] showed that the lesions produced in mice by the virus of encephalitis varies in intensity with the season. The cerebral reaction is of greatest severity in winter and of least severity in summer. Artificial raising or lowering of the temperature seemed to have the same effects. In similar experiments the intensity of the cerebral lesions of experimental typhus fever of the endemic type was also in inverse proportion to the temperature both seasonally and when artificially regulated. The clinical reaction and the duration of the disease were greater in winter than in summer, and the incubation period was longer in summer than in winter.

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126. Gilliam, A. G., and Decherd, G. M.: Note on the Preponderance of Cases with Bulbopontine Involvement in a Small Outbreak of Poliomyelitis in Austin, Texas, Pub. Health Rep. **52**:1853-1854 (Dec. 17) 1937.

127. Hudson, N. P., and Lennette, E. H.: Incidence of Poliocidal Services in Regions Where Poliomyelitis Epidemics Are Infrequent, Am. J. Trop. Med. **18**:35-40 (Jan.) 1938.

128. Greutter, J.; Brown, G. O.; Muether, R. O., and Casey, A. E.: Further Studies on the Virus of the 1937 Outbreak of Encephalitis in St. Louis, Proc. Soc. Exper. Biol. & Med. **37**:284-285 (Nov.) 1937. McCordock, H. A.; Smith, M. G., and Moore, E.: Isolation of St. Louis Encephalitis Virus During Inter and Epidemic Periods, ibid. **37**:288-290 (Nov.) 1937.

129. Howitt, B. F.: Antiviral Substances to the Virus of Encephalitis (St. Louis Type) in Serums Collected in California, Proc. Soc. Exper. Biol. & Med. **38**:334-336 (April) 1938.

130. Lillie, R. D.; Dyer, R. E.; Armstrong, C., and Pasternack, J. G.: Seasonal Variation in Intensity of Brain Reaction of the St. Louis Encephalitis in Mice and of Endemic Typhus in Guinea Pigs, Pub. Health Rep. **52**:1805-1822 (Dec. 10) 1937.

It is known that the virus of equine encephalomyelitis can be sedimented by ultracentrifugation. It has now been shown that the immune bodies contained in the tissues of animals which have recovered from infection can likewise be concentrated by high speed centrifugation.[131]

Lymphocytic Choriomeningitis.—Investigators[132] in the United States Public Health Service endeavored to determine how widely the virus of lymphocytic choriomeningitis is distributed in the United States by testing the serum of persons in different localities for the presence of specific protective bodies. Since recovery from the infection is followed by the production of demonstrable specific antibodies, it is assumed that persons whose serum is protective have actually suffered from the disease at some previous time. Protective antibodies were present in the serum from 138 (11 per cent) of the 1,248 persons tested. The serum of children rarely had protective antibodies. Positive results were obtained for serums that came from widely scattered areas of the country, and in many instances a history of meningeal symptoms could not be obtained. It is suggested that numerous cases of subclinical or undiagnosed choriomeningitis occur. Often the only symptoms present in proved cases are those easily mistaken for a cold or grip. Antibodies may persist in the blood for nearly four years after an attack.

The authors found it possible to transmit the infection to monkeys by instilling the virus into the urethra or the vagina. Virus has been found in the urine and in the seminal fluid of infected animals. These facts suggest the possibility of a venereal route as one mode of infection.

A serous form of meningitis of unknown etiology is described by Fatzer,[133] of Switzerland. The clinical description and laboratory findings are similar to those of lymphocytic choriomeningitis. It is regarded as a disease of young swineherds and is presumably contracted from sick pigs.

Measles.—Broadhurst and her associates[134] report the demonstration of inclusion bodies in the nasal mucous membranes and in the Koplik spots when preparations are stained with nigrosine. Inclusion bodies were said to be readily found in the mononuclear cells of the

131. Beard, J. W.; Finkelstein, H.; Sealy, W. C., and Wyckoff, R. W. G.: The Ultracentrifugal Concentration of the Immunizing Principle from Tissues Diseased with Equine Encephalomyelitis, Science **87**:89-90 (Jan. 28) 1938.

132. Wooley, J. G.; Armstrong, C., and Onstott, R. H.: The Occurrence in the Sera of Man and Monkeys of Protective Antibodies Against the Virus of Lymphocytic Choriomeningitis as Determined by the Serum-Virus Protection Test in Mice, Pub. Health Rep. **52**:1105-1114 (Aug. 13) 1937.

133. Fatzer, H.: Zur Frage der Meningitis serosa und der Maladie des jeunes Porchers, Schweiz. med. Wchnschr. **67**:709-712 (July 31) 1937.

134. Broadhurst, J.; MacLean, M. E., and Saurino, V. J.: Inclusion Bodies in Measles, J. Infect. Dis. **61**:201-207 (Sept.-Oct.) 1937.

blood when smears were stained with simple stains. They were present from one day before the exanthem broke out to ten days after its appearance. Inclusion bodies composed of rounded granules and compact crescentic bodies also appeared in tissue cultures of leukocytes taken from patients with measles on the third and the seventh day of the disease.

In another study,[135] cytoplasmic inclusion bodies obtained from patients with sore throat, chronic cough and other symptoms were also reported to be grown in tissue cultures. The cells containing such inclusion bodies do not show the same changes as those in cells of blood or tissue from patients with measles.

Foot and Mouth Disease.—An epizootic of foot and mouth disease broke out in Germany in 1937, as described by Wagener.[136] He also points out that there are three varieties of the virus, A, B and C, and that one type does not produce immunity against the others. Human beings may become infected; and after an incubation period of several days there is fever with a diphasic character, and primary blisters develop at the portal of entry of the virus, usually on the lips, mouth and hands. Etiologic diagnosis may be established by inoculating blister fluid into the scarified skin of the feet of guinea pigs.

Yellow Fever.—Soper and Smith[136a] found yellow fever virus grown in tissue cultures when mixed with human immune serum to be a safe and effective mixture for immunization but unpractical because of the small amounts available. A high percentage of failures occurred when persons were vaccinated with hyperimmune monkey serum and tissue culture virus. In 20 to 30 per cent of persons vaccinated with certain lots of vaccine, delayed reactions characterized by jaundice occurred within two to eight months. Vaccination against yellow fever is obviously not yet on a reliable basis.

The newly recognized jungle yellow fever of South America, which was at first believed to be transmitted to man by means other than the mosquito, has been transmitted by the Aedes aegypti mosquito under laboratory conditions.[136b]

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135. Broadhurst, J.; Cameron, G., and Saurino, V.: Measles Inclusion Bodies in Blood and in Tissue Culture, J. Infect. Dis. **62**:6-20 (Jan.-Feb.) 1938. Broadhurst, J.; Cameron, G., and Taylor, I.: Tissue Cultures of Human Throat Inclusion Bodies, ibid. **62**:21-26 (Jan.-Feb.) 1938.

136. Wagener, K.: Die Maul- und Klauenseuche als medizinisches Problem, Med. Klin. **34**:173-175 (Feb. 11) 1938.

136a. Soper, F. L., and Smith, H. H.: Yellow Fever Vaccination with Cultivated Virus and Immune and Hyperimmune Serum, Am. J. Trop. Med. **18**:111-134 (March) 1938.

136b. Whitman, L., and Antunes, P. C. A.: Transmission of Two Strains of Jungle Yellow Fever Virus by Aedes Aegypti, Am. J. Trop. Med. **18**:135-148 (March) 1938.

The Nature of Filtrable Virus.—Rawlins and Takahashi,[137] it seems, are not wholly convinced of the inanimate nature of certain viruses; at least they feel that the matter is by no means settled. Other observers have believed Stanley's crystals to be composed of elongated molecules in the liquid crystalline state. The molecules tend to come together with their long axes parallel, forming submicroscopic groups called swarms. The swarms presumably come together with their long axes parallel and are then visible. Under polarized light these liquid crystals may be indistinguishable from similar groups composed of submicroscopic crystals called tactoids. The virus of tobacco mosaic disease has some of the properties of liquid crystals and tactoids. Furthermore, the molecular weight of the tobacco mosaic virus protein lies between 10,000,000 and 17,000,000, and the weight of bacteriophage is probably over 200,000,000. The highest molecular weight of known enzymes is 82,800. Because of the wide differences in weight between the virus and the enzymes, the question is raised whether or not they are, after all, quite different. These and other puzzling facts provide opportunity for much more study of the subject.

MISCELLANEOUS DISEASES

Trichinosis.—Sawitz [138] made a study of the prevalence of trichinosis in the United States according to various authors who have made special studies of the subject in postmortem examinations. The incidence of trichinella infection was 12 per cent. Statistically the incidence of trichinosis has increased considerably in recent years, but this increase, as in other infectious diseases, more likely indicates that in more cases the condition is being correctly diagnosed. The mortality rate is lower than stated in other reports, which indicates that more mild cases are being recognized.

A large outbreak of trichinosis was observed [139] in a Civilian Conservation Corps camp in Vermont in October 1937. Forty-four patients required hospital treatment, and 20 remained ambulatory. All enrollees had been present at one meal and had eaten roast loin of pork which was said to have been undercooked in the center. The incubation period varied from three to twenty days, with an average of twelve days. Biopsy was made in several cases. In 1 case 800 larvae were estimated per gram of muscle. If larvae were distributed uniformly in this patient's musculature, it was estimated that over 26,000,000 were present. The

137. Rawlins, T. E., and Takahashi, W. N.: The Nature of Viruses, Science **87**:255-256 (March 18) 1938.

138. Sawitz, W.: Prevalence of Trichinosis in the United States, Pub. Health Rep. **53**:365-383 (March 11) 1938.

139. Ferenbaugh, T. L.; Segal, L., and Schulze, H. A.: A Trichinosis Epidemic of Sixty-Four Cases, J. A. M. A. **110**:1434-1436 (April 30) 1938.

remaining 136 healthy members of the company were given the cutaneous and the precipitin test; 20 gave positive reactions. Seven of the 20 had no symptoms of illness, 5 had been under observation for suspected trichinosis and 8 had the indefinite symptoms of a cold, with swollen conjunctivae, painful eyeballs, muscular weakness and malaise. In this epidemic, therefore, about one third of the infected persons were ambulatory, with subclinical infection.

One wonders how many patients thought to be suffering from colds actually have a mild form of some other disease. In the epidemic just reported mild trichinosis was the cause, and as stated elsewhere in this review, choriomeningitis was apparently the cause. A few years ago a number of persons in Brazil who supposedly had colds or influenza actually had yellow fever. In many cases poliomyelitis in its early stages is thought to be a cold. Pneumonic plague in California was thought to be influenza until an investigation was made. Many other examples of error could be cited.

Malaria.—Important experimental studies on malaria are under way in the laboratories of the International Health Division of the Rockefeller Foundation.[140] A certain degree of immunity to malaria is generally believed to exist after recovery from the disease. Serum from monkeys with chronic plasmodial infection, Coggeshall and Kumm found, was effective in reducing the intensity of the primary infection in other animals. Protective antibodies appear in the blood of monkeys during experimental infection. Coggeshall[141] inoculated monkeys with Plasmodium knowlesi and Plasmodium inui. As an immediate response, the spleen became enlarged, at times even before parasites appeared in the peripheral blood. The more virulent P. knowlesi caused less enlargement of the spleen than P. inui.

An interesting paper by Mayne[142] discusses the question of protracted incubation or latency of malarial infection. In 1 patient observed the latent period between the time of infection and the manifestation of symptoms was one year. Other observers also noted long periods, of six months or more. In considering the reason for the delay of evidence of disease, several factors may be operative: the condition of the patient, the season of the year when infection occurs, the size of the dose of infection and, of course, the variety of Plasmodium dealt with. The long latent period in malaria also may account for the development

140. Coggeshall, L. T., and Kumm, H. W.: Demonstration of Passive Immunity in Experimental Monkey Malaria, J. Exper. Med. **66**:177-189 (Aug.) 1937.

141. Coggeshall, L. T.: Splenomegaly in Experimental Monkey Malaria, Am. J. Trop. Med. **17**:605-617 (July) 1937.

142. Mayne, B.: Protracted Incubation in Malarial Fever, Pub. Health Rep. **52**:1599-1607 (Nov. 12) 1937.

of malaria in a recipient after transfusion of blood from a donor who has latent malaria. The long period of latency of malaria recalls the speculation of Zinsser regarding the latency of typhus fever. Jews living in eastern Europe, it is believed, were infected with the European form of typhus in childhood, but the infection remained latent for twenty or more years, long after immigration to America. In later life some circumstance permits the infection to become active and so-called Brill's disease results. I recently observed a similar example of a long latent period of kala-azar. A Chinese student had been in the United States for over six months when a typical attack of the disease occurred. Leishman-Donovan bodies were recovered from the blood and spleen, and the patient promptly recovered after treatment with an antimony preparation.

Papers given at a symposium on malaria by members of the National Malaria Committee were published in the August and September numbers of the *Southern Medical Journal*. Most of the papers were in the form of reviews of recent advances in the subject.

The Malaria Commission of the League of Nations made a study on the use of various synthetic drugs and quinine in the prevention and treatment of malaria. A report [143] of the commission contains the unanimous views of its members and practical recommendations for the treatment and prevention of the disease. Another valuable report was published by the League of Nations.[143a]

Rickettsial Diseases.—An excellent discussion of the varieties, epidemiology and geographic distribution of rickettsial diseases has been published by Zinsser [144] and on Rocky Mountain spotted fever by Parker.[144a]

Diphtheria.—Park [145] points out the difficulties inherent in deciding on the best method of immunization against diphtheria. The varying reports of the value of different procedures as found in the literature are confusing and probably result from the insufficient number of cases studied and from the use of different preparations. He outlines an ideal experiment to decide the matter. At present the best method of immunization, he believes, is the administration of two or three doses of either fluid or alum-precipitated toxoid.

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143. Therapeutics and Prophylaxis of Malaria, Miscellany, J. A. M. A. **110**: 1395-1397 (April 23) 1938.

143a. Fourth General Report of the Malaria Commission: The Treatment of Malaria, Bull. Health Organ., League of Nations **6**:897 (Dec.) 1937.

144. Zinsser, H.: The Rickettsia Diseases: Varieties, Epidemiology and Geographical Distribution, Am. J. Hyg. **25**:430-463 (May) 1937.

144a. Parker, R. R.: Rocky Mountain Spotted Fever, J. A. M. A. **110**:1185-1188 (April 9); 1273-1278 (April 16) 1938.

145. Park, W. H.: Duration of Immunity Against Diphtheria Achieved by Different Methods, J. A. M. A. **109**:1681-1683 (Nov. 20) 1937.

RARE AND "NEW" INFECTIONS OF ANIMALS AND MAN

Reports appear continually of rare or of presumably new diseases in animals and man which seem to complicate further and add to the long list of well known infections. On the other hand, certain diseases that are supposedly different, such as Oroya fever and Verrugas peruviana, and valley fever and coccidioidal granuloma, as described in a following paragraph, are shown to represent merely different stages of the same disease. Thus, as new methods are devised for diagnosis and new entities are delineated, the science of medicine actually becomes simplified. One questions the advisability of regarding many of the entities as "new" infections; they have probably existed unrecognized or have appeared at long intervals. It is safe to prophesy the recognition of many more infectious diseases as entities as studies are pursued further.

Whenever the discovery of a new form of micro-organism or virus in animals is announced, one immediately wonders whether or not similar forms may not be the cause of some infection in human beings of unknown origin. One is reminded of the brilliant results obtained from the studies of influenza in swine as related to influenza in human beings.

Coccidioidal Granuloma.—Dickson [146] cites evidence to show that the puzzling condition called valley fever, as it occurs in the San Joaquin Valley in California, is actually the first stage of the disease known as coccidioidal granuloma. Among 14 patients with coccidioidal granuloma, 3 had apparently had valley fever previously. Valley fever is characterized by acute pulmonary involvement, fever, cough and sputum. It is often mistaken for pulmonary tuberculosis until the shadows are found to disappear rapidly from the roentgenograms. Erythema nodosum commonly occurs. After an interval of several weeks the symptoms of coccidioidal granuloma appear. The author suggests that the initial infection occurs in the respiratory tract and that coccidioidal granuloma is a secondary manifestation resulting from hematogenous dissemination of the fungus after a period of latency.

Epidemic Diarrhea of the Newborn.—A series of outbreaks of a highly fatal unknown form of infectious diarrheal disease has affected newborn babies in hospital nurseries since 1934. In a statistical study [147] of 4,594 births, 711 cases of the disease are recorded, which gives a

146. Dickson, E. C.: "Valley Fever" of the San Joaquin Valley and Fungus Coccidioides, California & West. Med. **47**:151-155 (Sept.) 1937; Coccidioides Infection, Arch. Int. Med. **59**:1029-1044 (June) 1937.

147. Rice, J. L.; Best, W. H.; Frant, S., and Abramson, H.: Epidemic Diarrhea of the New-Born: I. Preliminary Considerations on Outbreaks of Highly Fatal Diarrhea of Undetermined Etiology Among New-Born Babies in Hospital Nurseries, J. A. M. A. **109**:475-481 (Aug. 14) 1937.

morbidity rate of about 15 per cent in the groups involved. Of these patients, 335 (47 per cent) died. Extensive study has failed to solve the problem of the etiology, and none of the usual methods of controlling epidemics has had any influence in stopping the infection. It is proposed to close nurseries and to suspend maternity services in places where infection is present in an attempt to stamp out the disease. The symptomatology, epidemiology, bacteriology, pathology and control measures are described in a paper by Best.[148] Bundesen [149] contrasts the regulations proposed by Best with those applied in Chicago.

Pemphigus.—Two investigators [150] report the development of illness and pathologic changes in irradiated mice after intracerebral inoculation of spinal fluid and fluid from blisters obtained from patients with pemphigus. The coat became rough two days after inoculation, ataxia and cachexia developed and death occurred. Histologically, necrotic areas were observed in the brain and in the meninges. The disease could be propagated by further transfer. Control animals showed no lesions.

American Trypanosomiasis.—Insects infected with Trypanosoma cruzi were found near Los Angeles by Wood.[151] The trypanosoma which is the cause of Chagas' disease in South America was first found in San Diego County and in Arizona several years ago by Kofoid.

Some French observers [152] reported an unusual infection incited by the bite of a rat. The patient's symptoms resembled those of the usual form of rat-bite fever, or sodoku, but differed in certain respects; there were sore throat, arthralgia and hydrarthrosis. A bacillus identified as Streptobacillus moniliformis was recovered from the blood. This bacillus occurs normally in the oral cavities of rats.

Spirochetal Jaundice.—Report [153] is made of a case of infection caused by Leptospira canicola characterized by meningeal symptoms and apparently contracted from a Samoyed dog. An epidemic apparently caused by Leptospira icterohaemorrhagicae and regarded as Weil's

148. Best, W. H.: Epidemic Diarrhea of the Newborn, J. A. M. A. **110**:1155-1158 (April 9) 1938.

149. Bundesen, H. N.: Regulations for Prevention of Epidemic Diarrhea of the Newborn, J. A. M. A. **110**:1301-1302 (April 16) 1938.

150. Grace, A. W., and Suskind, F. H.: An Agent, Transmissible to Mice, Obtained During a Study of Pemphigus Vulgaris, Proc. Soc. Exper. Biol. & Med. **37**:324-326 (Nov.) 1937.

151. Wood, S. F.: A New Locality for Trypanosoma Cruzi Chagas in California, Science **87**:366-367 (April 22) 1938.

152. Lemierre, A.; Reilly, J.; Laporte, A., and Morin, M.: Sur une nouvelle fièvre par morsure de rat, Bull. Acad. de méd., Paris **117**:705-713 (June 22) 1937.

153. Roos, C. J.; Walch-Sorgdrager, B., and Schüffer, W. A. P.: Epidemic of Leptospira Canicola Infection in Human Subjects and Dogs, Nederl. tijdschr. v. geneesk. **81**:3324-3335 (July 10) 1937.

disease, or infectious jaundice, involved 32 of 122 students who were probably exposed by contact with contaminated holy water in fonts.[154] Other reports of the disease have appeared from many parts of the world.[155] Weil's disease probably occurs more frequently than is believed in the United States, and efforts should be made to diagnose the condition correctly. The increase in the incidence of the disease is perhaps not due to its spread or to its actual increase but to correct diagnosis in cases in which the condition would have been mistaken for some other infection.

Four cases occurred in San Francisco in March 1937. Thirty-three per cent of the rats in one community were found to harbor the spirochetes. Meyer and his associates [156] encountered leptospiras from sick dogs in California and had difficulty in transmitting the infection to rodents. The micro-organisms were classed as L. canicola. Canine, murine and human leptospirosis, they believe, is not uncommon in California. Similar findings were reported from Germany.[157] Cases were reported from Detroit [157a] and Brooklyn.[157b] In Scotland the disease occurs among those who handle fish [157c] and tripe.[157d]

An unusual and hitherto unrecognized filtrable virus was encountered in rats by Woglom and Warren [158] during studies on rat sarcoma. The virus was apparently encountered accidentally. When injected subcutaneously into normal rats it causes a vigorous proliferation of connective tissue accompanied by necrosis and pus formation. The infected rats seldom show any signs of systemic disease, they do not lose weight and none dies. The effects are more serious after intravenous inoculation; redness, swelling and abscess formation occur in the

154. Willett, J. C.; Sigoloff, E., and Pfau, C. L.: An Institutional Outbreak of Epidemic Jaundice, J. A. M. A. **106**:1644-1646 (May 9) 1936.

155. Spirochetal Jaundice, editorial, J. A. M. A. **109**:1128-1130 (Oct. 2) 1937.

156. Meyer, K. F.; Eddie, B., and Anderson-Stewart, B.: Canine, Murine and Human Leptospirosis in California, Proc. Soc. Exper. Biol. & Med. **38**: 17-19 (Feb.) 1938.

157. Wirth, D.: Die Leptospirose (Weilsche Krankheit) bei Tieren, Wien. klin. Wchnschr. **50**:1115-1117 (July 30) 1937.

157a. Molner, J. G., and Kasper, J. A.: An Outbreak of Jaundice in Detroit, J. A. M. A. **110**:2069-2070 (June 18) 1938.

157b. Glotzer, S.: Weil's Disease: Report of a Case in a Fish Worker, J. A. M. A. **110**:2143-2145 (June 25) 1938.

157c. Davidson, L. S. P.: Weil's Disease: New Occupational Disease in Fish Workers, Glasgow M. J. **11**:113 (March) 1938.

157d. Stuart, R. D.: Leptospirosis in Glasgow Tripe Workers: Report of Possible Case of Leptospira Canicola Infection, Lancet **1**:603-606 (March 12) 1938.

158. Woglom, W. H., and Warren, J.: A Pyogenic Virus in the Rat, Science **87**:370-371 (April 22) 1938.

feet. The virus could be cultivated in the chorioallantoic membrane of the chick, and it retained its virulence during three transfers.

Nelson [159] reports an outbreak of a spontaneous disease of mice called infectious catarrh which is caused by a newly recognized micro-organism, a minute gram-negative coccobacillus, similar to that associated with fowl coryza.

An infectious disease of unknown etiology was observed in Australia by Derrick.[160] Workers in meat packing plants were involved. The onset of the illness was abrupt, and the chief complaints were of malaise, headache, pains and aches in the body, anorexia and fever. The face was flushed, headache was persistent, and drowsiness or stupor was common. Fever lasted from seven to twenty-four days. All the patients recovered. When the diagnosis was being made the usual forms of known infection were ruled out by appropriate tests. Guinea pigs could be infected by injecting blood or urine of a patient. They were immune to reinfection. No micro-organism has been recovered, but Burnet, who studied sections of tissues of animals experimentally infected, claims to have discovered rickettsia-like bodies in the spleen. The disease has been named Q fever.

MISCELLANEOUS

Certain interesting studies [161] recently threw some light on the long-suspected relation between the so-called fusiform and the spiral bacteria which normally inhabit the mouth. In studying colony formations of these bacteria, smooth colonies were found to be composed of short bacilli in the center, with spiral forms around the edge. The spiral forms appeared to be the rough dissociant culture phases of the bacilli.

Rogers [162] made some interesting studies of the possible changes which occur in the blood during acute paroxysms of fever. The increase in the density of the blood which precedes the fever, chill, muscle spasm, pallor and peripheral constriction was thought to be part of the anaphylactoid reaction of the fever-producing agent rather than an essential feature of the mechanism of fever itself. As fever sets in there is a

159. Nelson, J. B.: Infectious Catarrh of Mice, J. Exper. Med. **65**:833-842, 843-849 and 851-860 (June) 1937.

160. Derrick, E. H.: "Q" Fever, New Fever Entity: Clinical Features, Diagnosis and Laboratory Investigation, M. J. Australia **2**:281-298 (Aug. 21) 1937; A New Virus Disease, Foreign Letters, J. A. M. A. **110**:1765-1766 (May 21) 1938.

161. Tunnicliff, R., and Hammond, C.: Further Observations on Rough Cultures of Fusiform Bacilli, J. Infect. Dis. **61**:26-30 (July-Aug.) 1937.

162. Rogers, F. T.: Changes in Blood Density in Adult Man in Acute Fever Induced by Typhoid Vaccine and Malaria, Proc. Soc. Exper. Biol. & Med. **38**: 73-76 (Feb.) 1938.

temporary state of dilution of the blood, which returns to normal when the fever ends, especially after sweating.

A report of the Metropolitan Life Insurance Company [163] shows some striking features in the decline in incidence of certain infectious diseases during the past twenty years. The following table is based on experiences among policy holders of all ages:

Death Rates from Infectious Diseases, Showing the Greatest Percentage of Decline Between 1911-1915 and 1931-1935

	Percentage Decline
Typhoid fever	90.2
Malaria	87.8
Diphtheria	87.2
Diarrhea and enteritis	82.7
Measles	77.0
Whooping cough	71.7
Tuberculosis	70.2
Scarlet fever	69.2
Rheumatic fever	60.0
Erysipelas	59.3
Influenza and pneumonia	44.2

One is inclined to look for some explanation of these figures, since the incidence and mortality rates of influenza or of pneumonia, for example, in other statistics show that practically no change has occurred. It is also difficult to account for a 60 per cent reduction in death rate for rheumatic fever and erysipelas.

Application of the Langmuir Monolayer Film Technic to Biologic Problems.—Shaffer and Dingle [164] applied Langmuir's ingenious method of measuring molecular dimensions to the study of antigens and antibodies. They found, for example, that the adsorption of type III pneumococcus carbohydrate on a polished chromium surface coated with a multiple monomolecular layer of stearate yielded a film with a maximum thickness of 50 angstrom units. Antipneumococcus type III rabbit serum when applied to such an area increased the thickness to 100 angstrom units, and antipneumococcus type III horse serum increased it to 240 angstrom units. Experiments with other types of pneumococcus antigens and antibodies suggested that in the horse the size

163. Statist. Bull. Metropolitan Life Insur. Co. **18**:3-5 (Sept.) 1937.

164. Shaffer, M. F., and Dingle, J. H.: A Study of Antigens and Antibodies by the Monolayer Film Technique of Langmuir, Proc. Soc. Exper. Biol. & Med. **38**: 528-530 (May) 1938.

of the antibody protein molecule or aggregate varies according to the type of the pneumococcus, as found also by using different methods, such as passage through collodion membranes or by ultracentrifugation. The film method provides a promising means for further investigation of biologic problems.

Ecker and his associates [165] discovered the important fact that a definite relation exists between the amount of vitamin C in the diet and the titer of complement of the serum of guinea pigs. A low complement titer resulting from vitamin C deficiency could be increased by adding crystalline vitamin C to the diet. Ascorbic acid seemed to be essential to the development of complement.

165. Ecker, E. E.; Pillemer, L.; Wertheimer, D., and Gradis, H.: Ascorbic Acid and Complement Function, J. Immunol. **34**:19-38 (Jan.) 1938.

Book Reviews

A Primer for Diabetic Patients. By Russell M. Wilder, M.D., Professor and Chief of the Department of Medicine of the Mayo Foundation, University of Minnesota. Price, $1.75. Pp. 191, with 1 colored plate, 3 illustrations and many tables. Philadelphia: W. B. Saunders Company, 1937.

This is an illuminating little book for any one to study who has a doubting mind about medical progress, for it depicts so well how knowledge advances.

The first edition appeared in 1921, an offshoot of what the author felt was "the need for a brief outline of the principles underlying the dietary treatment of diabetes in the daily instruction of patients in the matter of their diets and hygiene." It was at first a simple affair; a mimeographed outline. But the patients liked it and asked for more, and hence in due time the primer appeared in book form, attractive looking and full of common sense. New editions were necessary every two or three years. The popularity of the "Primer" is a good indicator of the fact that patients with diabetes wish to keep abreast of the times and try to be a jump or two ahead of their disease. Each new edition was a little larger, a little more pretentious and a little fuller of information than its predecessor, but equally well liked. The first edition was 76 pages long, the second 119, the fourth 138 and the fifth 172; and now the sixth has 191 pages to its credit. It probably is no exaggeration to say that the diabetic patient in 1938 must know twice as much about his illness in order to remain well as he did in 1921 and that there is twice as much knowledge available for him to acquire. Now, in addition to everything else that he must be taught in regard to diets, urinalyses and the prevention of coma and gangrene, he must learn about protamine zinc insulin, how to use it and how it differs from old insulin. All this is told in the new "Primer" with charming simplicity and honesty.

A sister of the ARCHIVES, *The Journal of the American Medical Association* (J. A. M. A. **82**:572 [Feb. 16] 1924; **96**:553 [Feb. 14] 1931; **103**:1976 [Dec. 22] 1934), on several occasions has had nice things to say about the "Primer." The ARCHIVES, too, would like to praise it. For, as unpretentiously as ever, the "Primer" continues to be what it started out as: a brief, well written, authoritative outline of the principles underlying the modern treatment of diabetes expressed in easily understood English. Doctor as well as patient should read it, lest the patient on visiting the doctor demonstrate that of the pair he is the more alert and better educated diabetically.

The Larynx and Its Diseases. By Chevalier Jackson, M.D., and Chevalier L. Jackson, M.D. Price $8. Pp. 555, with 220 illustrations, including 11 colored plates. Philadelphia: W. B. Saunders Company, 1937.

This book will receive and certainly deserves a hearty welcome from the members of the medical profession in this country. Those interested in laryngology in particular have looked forward to its publication. It is the only textbook of recent years devoted exclusively to the diseases of the larynx. As the authors say in their preface, "This is remarkable when one considers the prevalence of laryngeal disease."

The book itself is not too voluminous, and it does not tire one with insignificant details. Essentially, it is a clear, concise review of all the diseases peculiar to the larynx. In addition, it contains a full chapter relative to the laryngeal phases of general disease. In this respect, and in many others too numerous to mention, the book should be a valuable adjunct to the library of the general practitioner.

The varied phases of laryngeal disturbances are thoroughly presented in an orderly fashion. For each disease the authors give the definition and then present the incidence, etiology, pathology, laryngeal appearance, symptomatology, diagnosis, complications, treatment, prognosis and sequelae and a historical note. The

last four chapters are devoted to the description of laryngeal and endolaryngeal operative procedures.

There are two hundred and twenty illustrations, including eleven plates in color, all of which were drawn by Dr. Chevalier Jackson.

All recent advances have been incorporated in this volume. It represents the current ideas in laryngology and will do much to bring forth more accurate diagnoses and a better understanding of methods of treatment. As a practical and useful textbook, this volume cannot be too highly praised.

Practical Proctology. By Louis A. Buie, M.D., Professor of Proctology, the Mayo Foundation. Price, $6.50. Pp. 512, with 152 illustrations. Philadelphia: W. B. Saunders Company, 1937.

This book is written with gusto and is a fine, enthusiastic achievement. It bears the hallmarks of Mayo Clinic efficiency. It is based on ten years' work in a most active medical center and on a personal experience of over forty thousand proctoscopic examinations. Needless to say, it is well printed and clearly illustrated.

Its title gives the clue to the character of the volume. It is not a treatise for the specialist; but, on the other hand, it is the kind of practical book that any internist will enjoy reading. It will make him realize that he can learn proctoscopic technic; that such examinations are important and that without ability to examine the lower portion of the bowel properly he is an unfinished diagnostician.

There is an excellent bibliography at the close of each chapter. Sensible diet lists are appended that may be useful in the treatment of intestinal conditions requiring high or low residues. There are practical prescriptions of one kind or another for palliative therapy as well as descriptions of formidable-sounding operative procedures that are now familiar to the expert proctologist.

In brief, one can here obtain a bird's-eye view of what modern proctology is; and if one wishes detailed information in any specific phase of the subject, the information is at hand, clearly expressed and always based on a palatable combination of experience and knowledge of the literature. On the whole, the book is admirable.

The Cerebrospinal Fluid. By H. Houston Merritt, M.D., and Frank Fremont-Smith, M.D. With a foreword by James B. Ayer, M.D. Price, $5. Pp. 333, with 17 illustrations and 63 tables. Philadelphia: W. B. Saunders Company, 1937.

This is a well assembled monograph. As Dr. Ayer states in the foreword, its purpose is to present facts—facts based on thousands of examinations of spinal fluid made under standard conditions in connection with a variety of diseased states in the wards and in the laboratory of the Boston City Hospital.

The book is written in a scholarly fashion, and due respect is paid to the literature. Nearly eight hundred references are mentioned which have been assembled since Quincke began the clinical examination of spinal fluid, in 1891. And so, throughout the entire volume, the writers compare their own experience with that of others and build up a notably comprehensive and reliable account of the present position of this particular laboratory test as a part of the diagnostic armamentarium.

The book is easily read as it is well and clearly written. The technic is clearly described, not merely the technic of how to withdraw spinal or cisternal fluid and how to measure the pressure properly, but also the technic of roentgenography of the ventriculosubarachnoid space (by Dr. T. J. C. von Storch) and the technic of cell counting and chemical determinations of the spinal fluid. The latter subjects were written about by Miss Mary D. Irvine, who must have had vast experience with these methods.

On the whole, here is a successful effort. It is an up-to-date account of the spinal fluid and will soon become a useful handbook in all hospitals and medical schools.